

## SUPPLEMENTARY INFORMATION

### Ion-pair Recognition by a Neutral [2]Rotaxane Based on a Bis-calix[4]pyrrole Cyclic Component

J. Ramón Romero,<sup>a</sup> Gemma Aragay,<sup>a</sup> Pablo Ballester<sup>\*a,b</sup>

<sup>a</sup>*Institute of Chemical Research of Catalonia (ICIQ), The Barcelona Institute of Science and Technology, Avda. Països Catalans 16, 43007, Tarragona, Spain.*

<sup>b</sup>*ICREA, Passeig Lluís Companys, 23, 08018 Barcelona, Spain.*

[pballester@iciq.es](mailto:pballester@iciq.es)

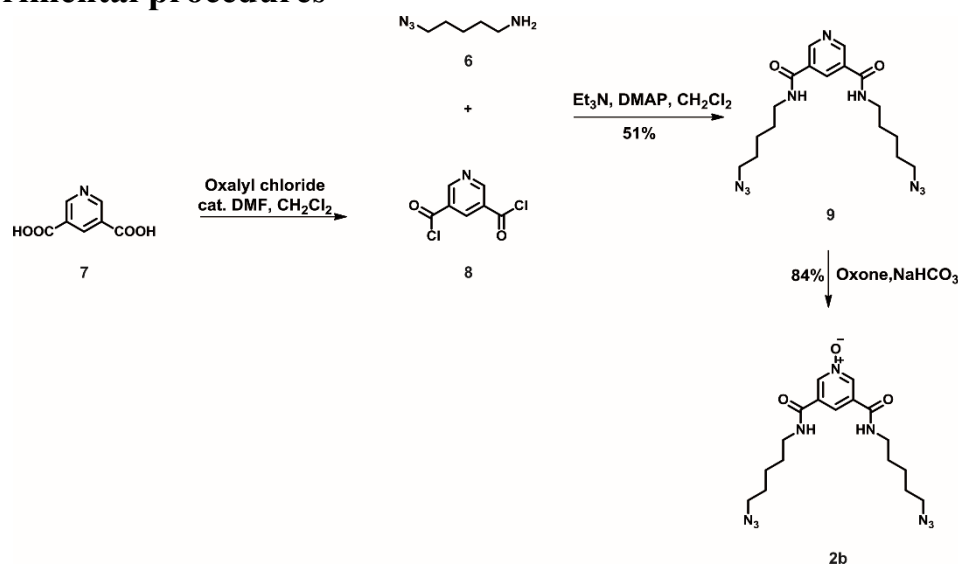
#### Contents

|                                                                         |     |
|-------------------------------------------------------------------------|-----|
| General information and instrumentation.....                            | S2  |
| Experimental procedures.....                                            | S2  |
| HPLC analysis.....                                                      | S7  |
| <sup>1</sup> H NMR spectra .....                                        | S8  |
| [2]Rotaxane Characterization.....                                       | S9  |
| <sup>1</sup> H NMR titrations of [2]rotaxane in CDCl <sub>3</sub> ..... | S16 |
| Isothermal Titration Calorimetry (ITC) .....                            | S24 |
| Simulated speciation profiles .....                                     | S26 |
| References .....                                                        | S27 |

## General information and instrumentation

All reagents were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from a solvent purification system SPS-400-6 from Innovative Technologies, Inc. All solvents were of HPLC grade quality, commercially obtained and used without further purification. Routine  $^1\text{H}$  NMR spectra were recorded on a Bruker Avance II 400 Ultrashield NMR spectrometer. Variable temperature experiments and 2D NMR spectra were performed on a Bruker Avance 500 (500.1 MHz for  $^1\text{H}$  NMR) Ultrashield spectrometer.  $\text{CDCl}_3$  from Sigma Aldrich was used for NMR studies. Chemical shifts are given in ppm, relative to TMS.

## Experimental procedures



Scheme S 1. Synthetic scheme for the preparation of *N*-oxide (**2b**). 5-azidopentan-1-amine (**6**) was synthesized following a reported procedure.<sup>1,2</sup>

### 3,5-bis((5-azidopentyl)carbamoyl)pyridine **9**

Compound **9** was prepared from commercially available 3,5-pyridinedicarboxylic acid by reaction with oxalyl chloride and a catalytic amount of DMF to obtain the corresponding acyl chloride **8**. To a solution of **8** in DCM (1.2 mM, 10 mL) a solution of 5-azidopentan-1-amine **6**<sup>3</sup> (1.53 g, 11.9 mmol),  $\text{Et}_3\text{N}$  (2.8 mL) and DMAP (0.69 g, 5.7 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (5 mL) was added dropwise at  $0^\circ\text{C}$ . The reaction mixture was stirred at room temperature for 2 h. After 2 hours, the reaction crude was washed with 0.01 N HCl (2x10 mL) followed by washes of saturated  $\text{NaHCO}_3$  (2x10 mL). The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. Purification of the residue by column chromatography (AcOEt as eluent) afforded **9** as yellow solid (1.13 g, 51 %).

$^1\text{H}$  NMR (400 MHz, Chloroform-*d*)  $\delta$  9.08 (s, 2H), 8.44 (s, 1H), 6.65 (br, 2H), 3.51 (m, 4H), 3.30 (m, 4H), 1.67 (m, 8H), 1.48 (m, 4H).

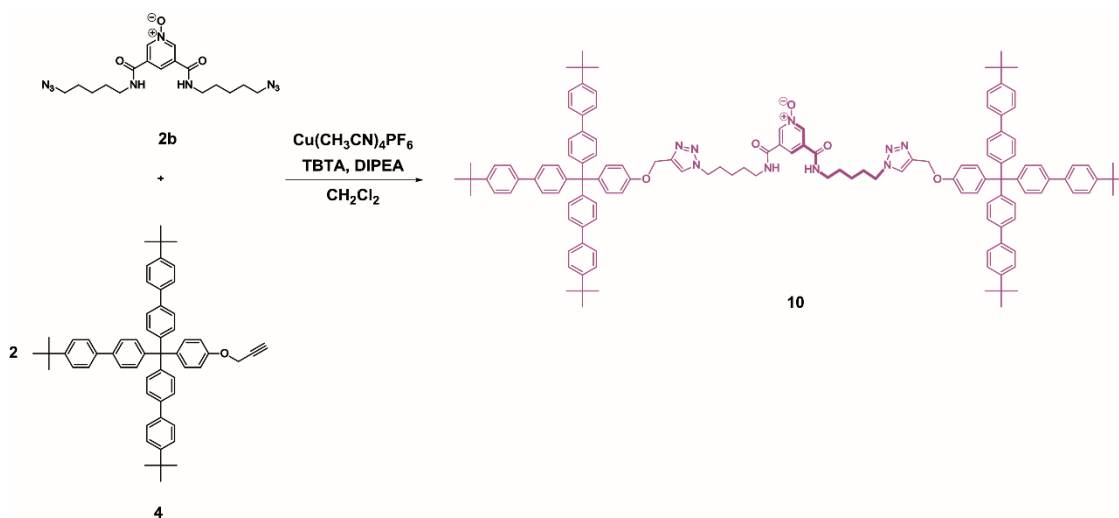
### 3,5-bis((5-azidopentyl)carbamoyl)pyridine 1-oxide **2b**

In a round bottom flask (250 mL) 3,5-bis((5-azidopentyl)carbamoyl)pyridine **9** (1.1 g, 2.9 mmol) and sodium hydrogencarbonate (7.4 g, 87.5 mmol) were dissolved in 30 mL mixture of

H<sub>2</sub>O/2-butanone 1:1. The solution was stirred vigorously for 5 min. Oxone was added dropwise to the solution (7 g in 10 mL of water). After 4 hours, the reaction mixture was treated with brine (20 mL) and the desired product was extracted with CHCl<sub>3</sub> (2x10 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to obtain a yellow solid (993 mg, 84%).

<sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.87 (s, 2H), 8.10 (s, 1H), 7.14 (br, 2H), 3.51 (m, 4H), 3.33 (m, 4H), 1.70 (m, 8H), 1.52 (m, 4H). <sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, Chloroform-*d*) δ 162.4, 140.3, 134.2, 124.8, 51.4, 40.5, 29.0, 28.7, 24.3. IR ν<sub>max</sub>/cm<sup>-1</sup>: 2935 (C-H, st), 2090 (N=N, st), 1645 (C=O, st), 1543 (N-O, st). HR-MS (ESI-TOF ES+) *m/z* calculated for C<sub>17</sub>H<sub>25</sub>N<sub>9</sub>O<sub>3</sub> ([M+H])<sup>+</sup> 404.2153 found ([M+H])<sup>+</sup> 404.2145

#### Linear Component **10**



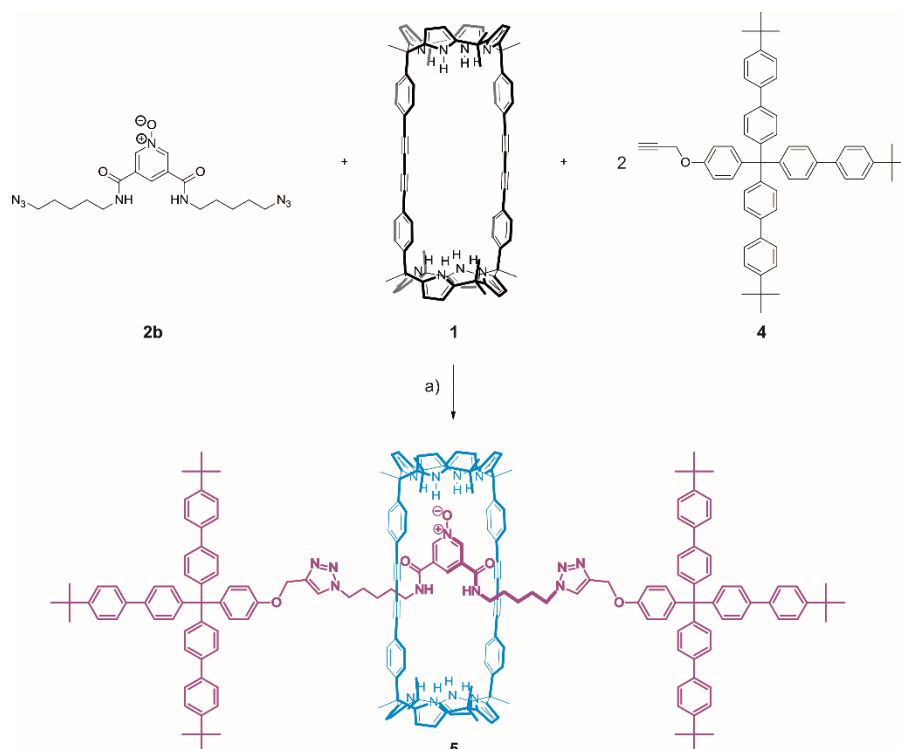
Scheme S 2. Synthetic scheme for the preparation of the linear component (**10**). Stopper (**4**) was synthesized following a reported procedure.<sup>4</sup>

A mixture of **2b** (32 mg, 0.08 mmol), **4** (127 mg, 0.16 mmol), Cu(CH<sub>3</sub>CN)<sub>4</sub>PF<sub>6</sub> (30 mg, 0.08 mmol) and TBTA (43 mg, 0.08 mmol) were dissolved in dry DCM (4 mL) in a two necked round bottom flask (50 mL). Finally, *N*-ethyl-*N*-isopropylpropan-2-amine (0.056 mL, 0.32 mmol) was added to the reaction mixture. The reaction was stirred at room temperature under argon for 3 hours. After 3 hours, the crude was washed with water (2x40 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The product was purified by silica column chromatography using DCM as eluent in order to collect the excess of stopper **4**. The eluent was changed to 5% MeOH/DCM to collect the desired product as a white solid (85 mg, 54%).

<sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 8.78 (s, 2H), 8.1 (br, 1H), 8.09 (s, 2H), 7.53 (d, *J* = 8.4 Hz, 12H), 7.52 (s, 2H), 7.49 (d, *J* = 8.4 Hz, 12H), 7.43 (d, *J* = 8.4 Hz, 12H), 7.29 (d, *J* = 8.4 Hz, 12H), 7.22 (d, *J* = 8.8 Hz, 4H), 6.8 (d, *J* = 8.8 Hz, 4H), 5.08 (s, 4H), 4.35 (m, 4H), 3.4 (m, 4H), 1.84 (m, 4H), 1.64 (m, 4H), 1.34 (s, 54H, CH<sub>3</sub>-*t*But), 1.28 (m, 4H). <sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, Chloroform-*d*) δ 162.8, 156.3, 150.4, 145.7, 144.4, 141.2, 140.9, 140.1, 138.6, 137.8, 132.5, 131.7, 126.7, 126.2, 125.8, 123.3, 121.5, 113.6, 64.0, 61.8, 49.8, 39.6, 34.8, 31.5, 29.5, 27.7, 22.8. IR ν<sub>max</sub>/cm<sup>-1</sup>: 2959 (C-H, st), 2867 (C-H, st), 1494 (C=N, C=C, st), 815 (C=C, st). HR-

MS (ESI-TOF ES+)  $m/z$  calculated for  $C_{133}H_{141}N_9O_3Na$  ( $[M+Na]^+$ ) 1967.0948 found ( $[M+Na]^+$ ) 1967.0938

[2]Rotaxane **5**



Scheme S 3. Synthetic scheme for the preparation of [2]rotaxane **5**. Macrocycle **1** was synthesized following a reported procedure.<sup>5</sup> a) Reaction conditions:  $[Cu[(CH_3CN)_4]PF_6]$ , TBTA, DIPEA, DCM.

**Conditions a:** Macrocycle **1** (21.1 mg, 0.018 mmol), pyridine *N*-oxide **2b** (7.1 mg, 0.018 mmol), **4** (27.2 mg, 0.035 mmol), TBTA (0.50 mg,  $0.9 \times 10^{-3}$  mmol) and the catalyst  $Cu[(CH_3CN)_4]PF_6$  (0.34 mg,  $0.9 \times 10^{-3}$  mmol) were placed in a two necked round bottom flask (25 mL) and dissolved in dry DCM (7 mL). Finally, the Hünig's base (0.012 mL, 0.070 mmol) was added to the reaction mixture and was stirred at room temperature under Ar for 5 hours. The reaction could be monitored by TLC (2% MeOH/DCM,  $R_f$  rotaxane=0.36;  $R_f$  macrocycle=1;  $R_f$  linear component=0). The reaction mixture was washed with water (2×40 mL). The organic phase was dried over anhydrous  $Na_2SO_4$  and the solvent was removed *in vacuo*. The residue was purified by column chromatography (30% AcOEt/DCM) to give the desired product as a white solid (6 mg, 11%). Upon changing the eluent to 5% MeOH/DCM we could isolate the free linear component.

**Conditions b** (using MTOACl as template): In a two necked round bottom flask (25 mL) macrocycle **1** (21.1 mg, 0.018 mmol), pyridine *N*-oxide **2b** (7.1 mg, 0.018 mmol), MTOACl **3d** (7.3 mg, 0.018 mmol) were dissolved in dry DCM (7 mL). The formation of the 1:1:1 **1:2:3d** pseudorotaxane complex was monitored by  $^1H$  NMR. After confirming the exclusive formation of the 1:1:1 complex in solution, **4** (27.2 mg, 0.035 mmol), TBTA (0.50 mg,  $0.9 \times 10^{-3}$  mmol) and the catalyst  $Cu[(CH_3CN)_4]PF_6$  (0.34 mg,  $0.9 \times 10^{-3}$  mmol) were added to the reaction

mixture. Finally, Hünig's base (0.012 ml, 0.070 mmol) was added and the reaction mixture was stirred at room temperature under Ar for 5 hours. After 5 hours the reaction crude was washed with water (2×40 mL). The organic phase was collected and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed *in vacuo*. The crude was purified by silica column chromatography, using 30% AcOEt/DCM as eluent. Different fractions were collected but none of them contained the pure free rotaxane but mixtures of the free macrocycle **1**, free linear component **10** and the ion-paired complex **3**–**5**. Other separation mixtures (e.g. MeOH:DCM 5:95) were also tried without separation success.

**Conditions c** (higher concentration of the reactants): In a two necked round bottom flask (25 mL) macrocycle **1** (100 mg, 0.084 mmol), pyridine *N*-oxide **2b** (33.7 mg, 0.084 mmol), **4** (129 mg, 0.167 mmol), TBTA (2.2 mg, 4.2×10<sup>-3</sup> mmol) and the catalyst Cu[(CH<sub>3</sub>CN)<sub>4</sub>PF<sub>6</sub>] (1.5 mg, 4.2×10<sup>-3</sup> mmol) were dissolved in dry DCM (6 mL). Finally, Hünig's base (0.06 ml, 0.33 mmol) was added to the reaction mixture. The reaction was stirred at room temperature under Ar for 5 hour. After 5 hours, the reaction crude was washed with water (2×40 mL). The organic phase was collected and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed *in vacuo*. The residue was purified by column chromatography (30% AcOEt/DCM) to give pure [2]rotaxane **5** as a white solid (72 mg, 27%).).

**Conditions d** (higher **1**:**2**:**4** molar ratios): In a two necked round bottom flask (25 mL) macrocycle **1** (100 mg, 0.084 mmol), pyridine *N*-oxide **2b** (67.4 mg, 0.167 mmol), **4** (258 mg, 0.334 mmol), TBTA (22 mg, 0.04 mmol) and the catalyst Cu[(CH<sub>3</sub>CN)<sub>4</sub>PF<sub>6</sub>] (16 mg, 0.04 mmol) were dissolved in dry DCM (10 mL). Finally, Hünig's base (0.1 ml, 0.7 mmol) was added to the reaction mixture. The reaction was stirred at room temperature under Ar for 5 hours. After 5 hours the reaction crude was washed with water (2×40 mL). The organic phase was collected and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed *in vacuo*. The residue was purified by column chromatography (30% AcOEt/DCM) to give pure [2]rotaxane **5** as a white solid (130 mg, 50%).).

<sup>1</sup>H NMR (500 MHz, Chloroform-*d*) δ 8.73 (br, 8H, NHe,f), 8.20 (s, 1H, H2), 7.64 (s, 2H, H9), 7.54 (d, *J* = 8.5 Hz, 12H, H15), 7.49 (d, *J* = 8.5 Hz, 12H, H16), 7.44 (d, *J* = 8.5 Hz, 12H, H14), 7.29 (d, *J* = 8.5 Hz, 12H, H13), 7.24 (m, 4H, H12), 6.96 (br, 8H, Hb, Hd), 6.88 (m, 12H, H11, Ha, Hc), 5.93 (m, 16H, Hg-j), 4.97 (br, 4H, H10), 4.18 (m, 4H, H8), 3.6 (br, 4H, H4), 1.92 (m, 4H, H7), 1.75 (br, 16H, H5, Hk-l), 1.55 (br, 24H, Hm,n), 1.35 (s, 54H, H17). <sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, Chloroform-*d*) δ 162.3, 156.2, 150.2, 149.6, 145.7, 143.9, 139.7, 139.3, 138.8, 138.4, 137.7, 135.9, 133.5, 132.3, 131.6, 131.4, 127.1, 126.6, 126.1, 125.9, 125.7, 123.9, 118.9, 113.4, 105.5, 102.9, 81.6, 74.2, 63.7, 61.3, 49.9, 45.4, 39.3, 34.8, 34.5, 31.5, 31.4, 29.8, 29.7, 28.8, 27.9, 22.9. IR ν<sub>max</sub>/cm<sup>-1</sup>: 3344 (N-H, st), 2960 (C-H, st), 2867 (C-H, st), 1494(C=N, C=C, st), 814 (C=C, st), 767 (C-H, δ oop). HR-MS (ESI-TOF ES+) *m/z* calculated for C<sub>217</sub>H<sub>217</sub>N<sub>17</sub>O<sub>5</sub>Na<sub>2</sub> ([M+2Na])<sup>2+</sup> 1593.3517, found ([M+2Na])<sup>2+</sup> 1593.3474

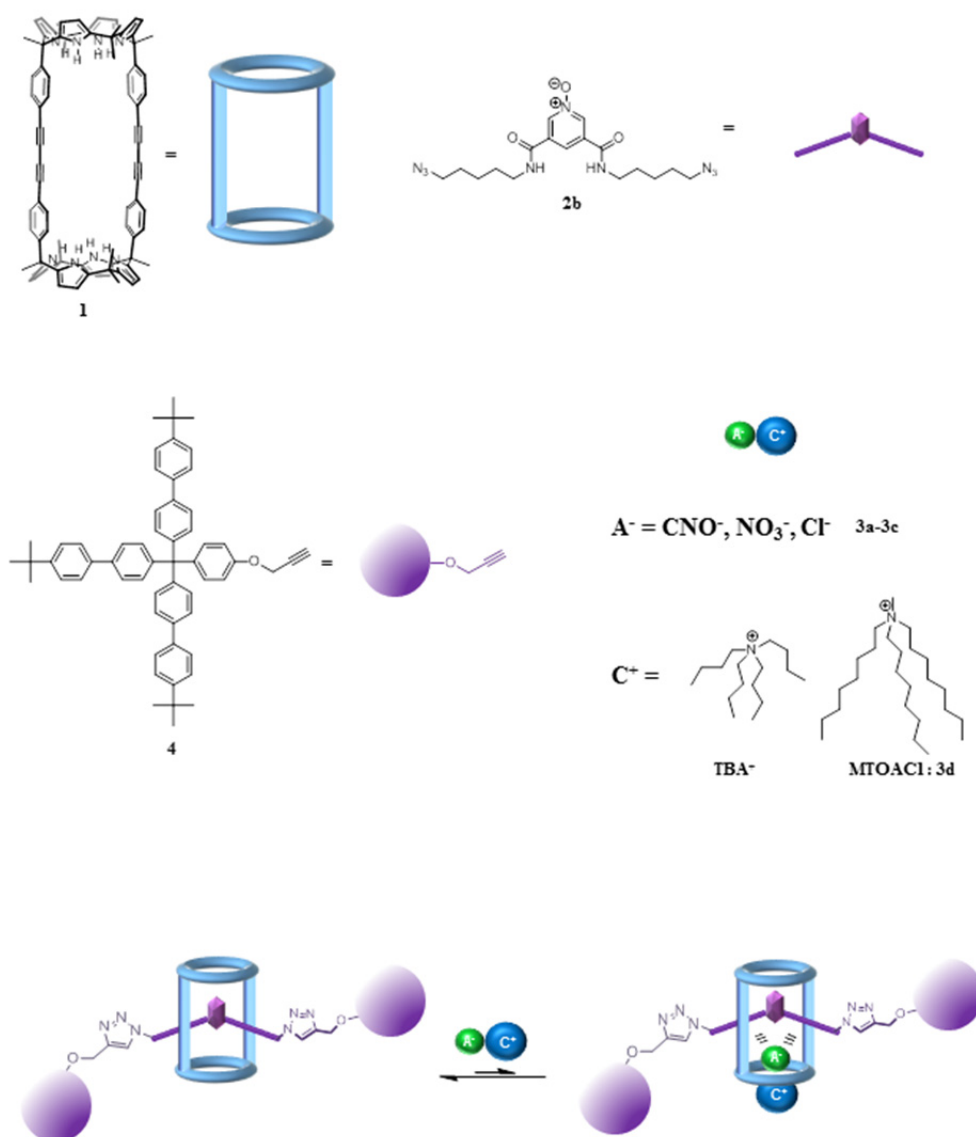


Figure S 1. Molecular structures of macrocycle **1**, bis-amidepyridyl-*N*-oxide axle **2b**, stoppers **4** and the tetraalkylammonium ion-pairs **3a-d** used in the work. The schematic representation used in the supporting information document for the individual molecular components and the supramolecular aggregates is also shown.

## HPLC analysis

HPLC experiments were performed using a HPLC1100 Agilent instrument. Chromatographic parameters: waters spherisorb 5 $\mu$  silica 4.6 mm x 250 mm column, 7% THF/DCM as mobile phase, flow rate 1 mL/min, T = 25  $^{\circ}$ C, injection volume 1 $\mu$ L.

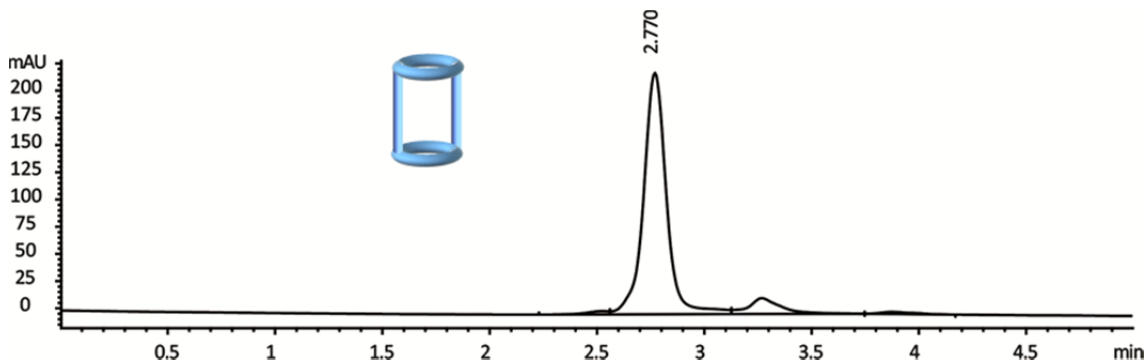


Figure S 2. HPLC trace of a 1 mM solution of pure macrocycle **1**.

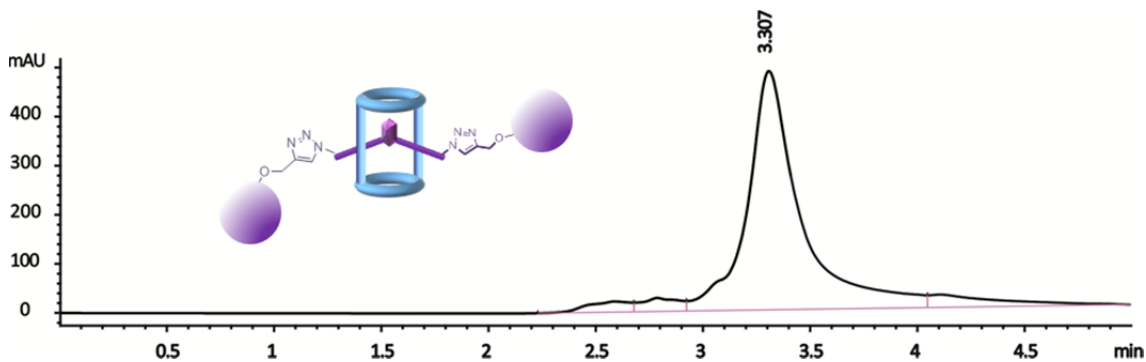


Figure S 3. HPLC trace of a 1 mM solution of pure [2]rotaxane **5**.

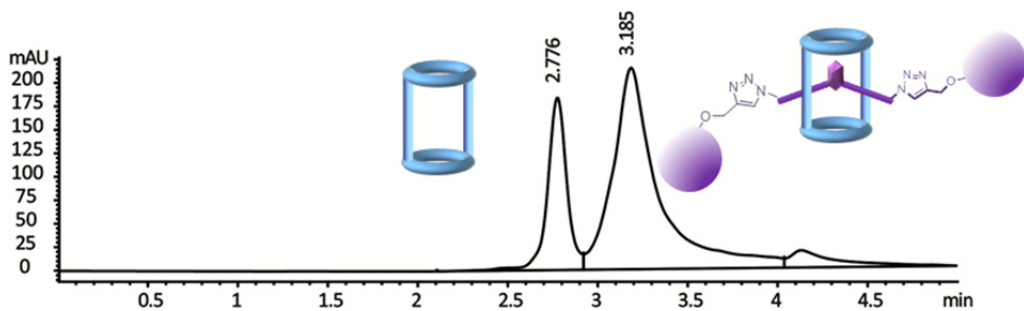


Figure S 4. HPLC trace of a 1 mM solution of an equimolar mixture [2]rotaxane **5** and macrocycle **1**.

## <sup>1</sup>H NMR spectra

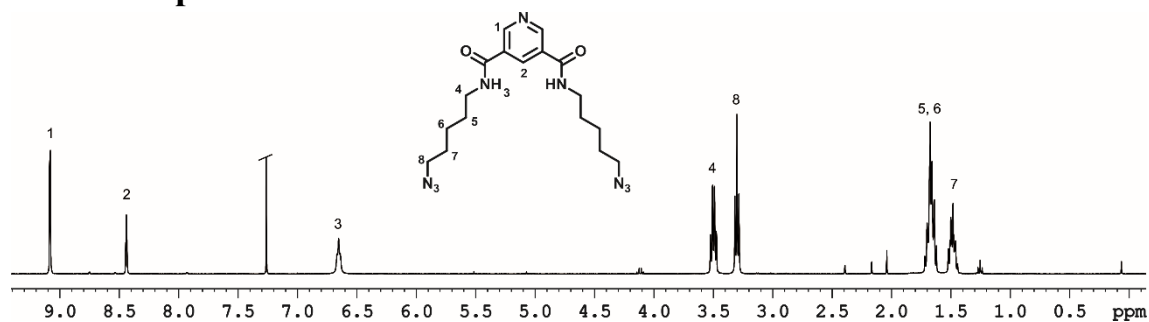


Figure S 5. <sup>1</sup>H NMR spectrum of **9** (CDCl<sub>3</sub>, 400MHz, 298K) with the corresponding proton assignment.

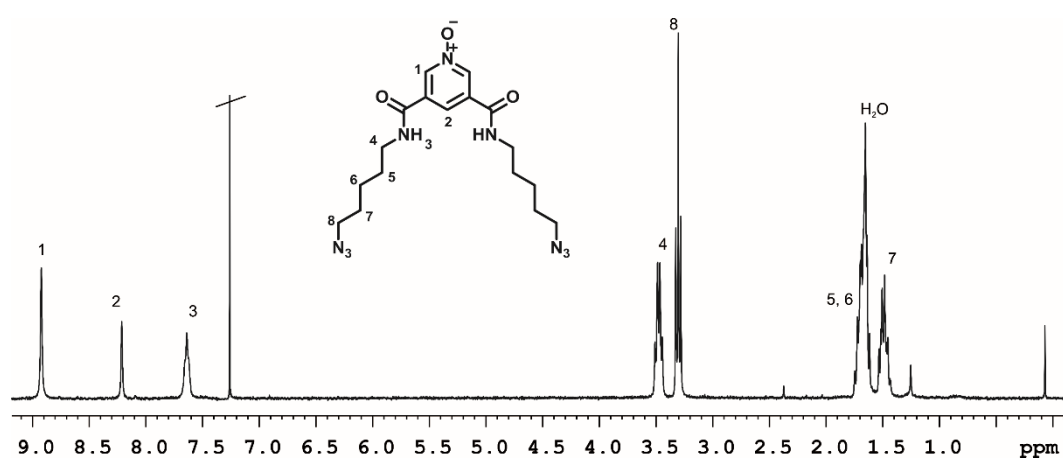


Figure S 6. <sup>1</sup>H NMR spectrum of N-oxide **2b** (CDCl<sub>3</sub>, 400MHz, 298K) with the corresponding proton assignment.

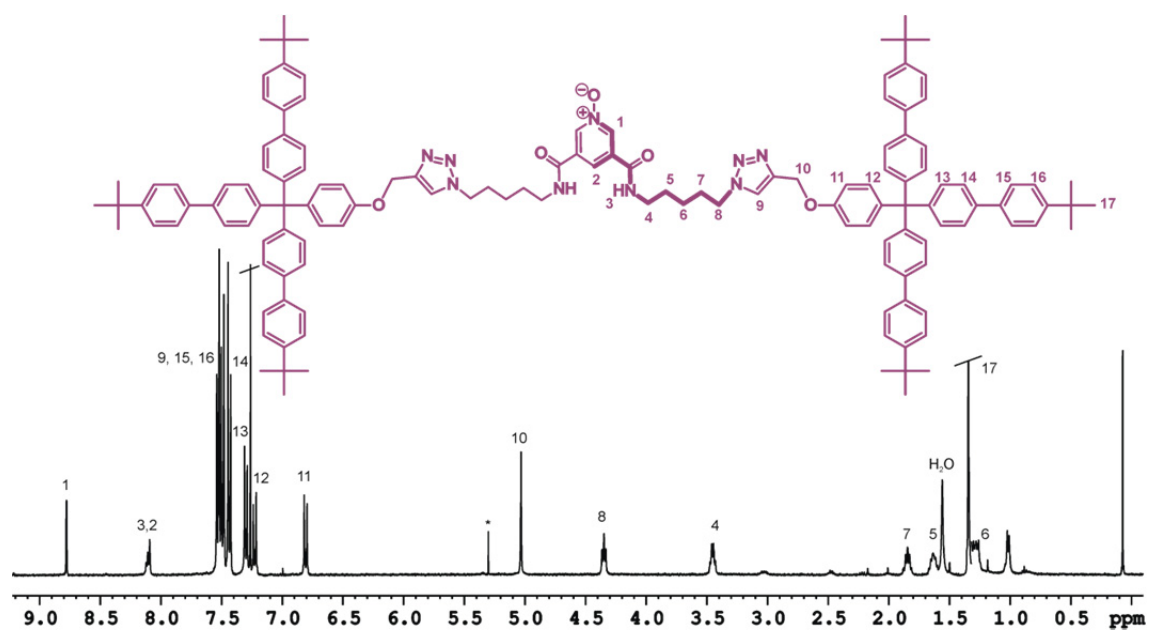


Figure S 7. <sup>1</sup>H NMR spectrum of linear component **10** (CDCl<sub>3</sub>, 400MHz, 298K) with the corresponding proton assignment.\* solvent signal.

## [2]Rotaxane Characterization

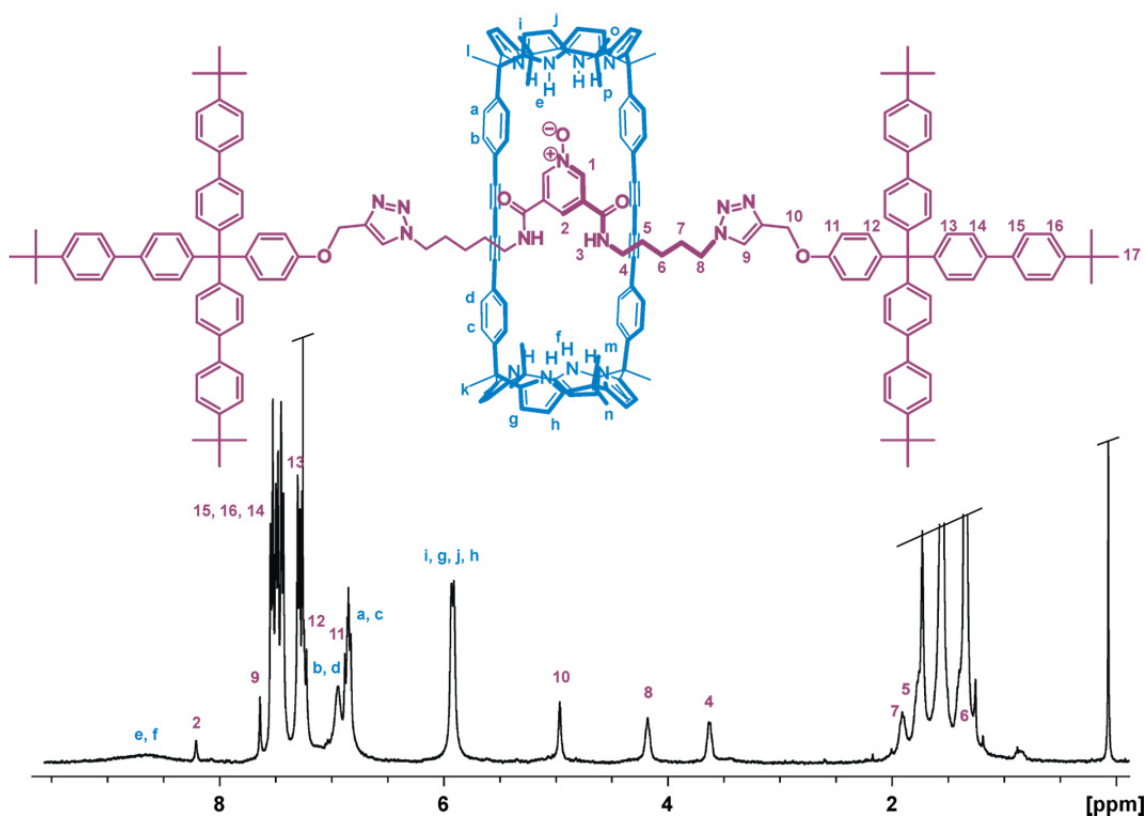


Figure S 8.  $^1\text{H}$  NMR spectrum of a 1 mM solution of [2]rotaxane **5** ( $\text{CDCl}_3$ , 500MHz, 298K) with the corresponding proton assignment.

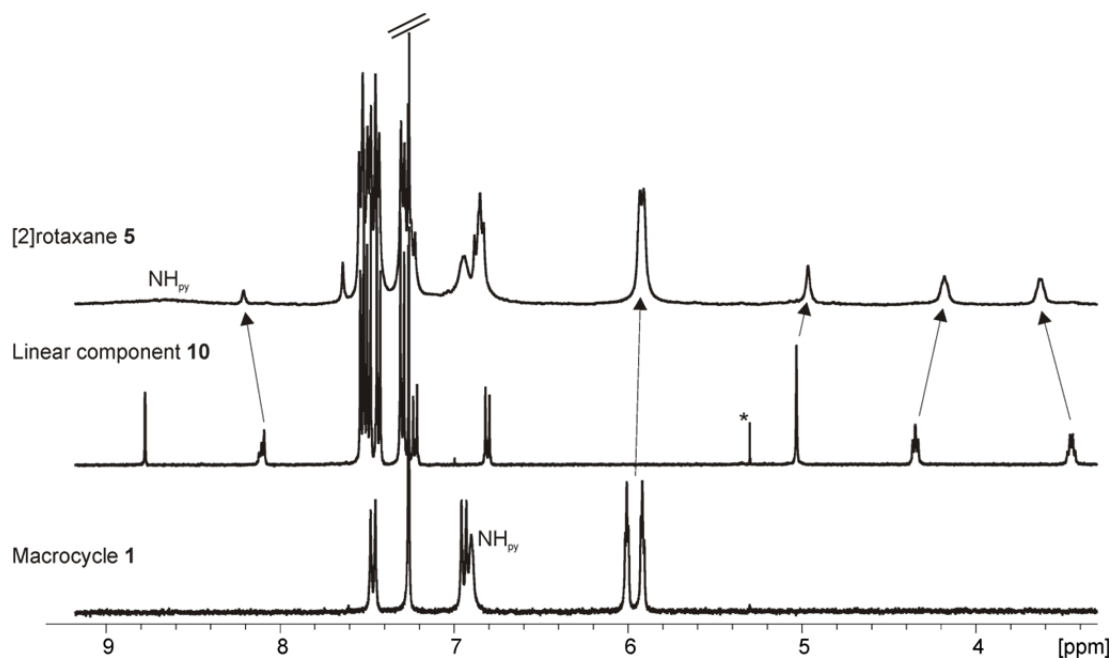


Figure S 9. Selected downfield regions of the  $^1\text{H}$  NMR spectra (500 MHz,  $\text{CDCl}_3$ , 298 K) of the free cyclic (**1**) and linear (**10**) components and [2]rotaxane **5**. (\*solvent peak)

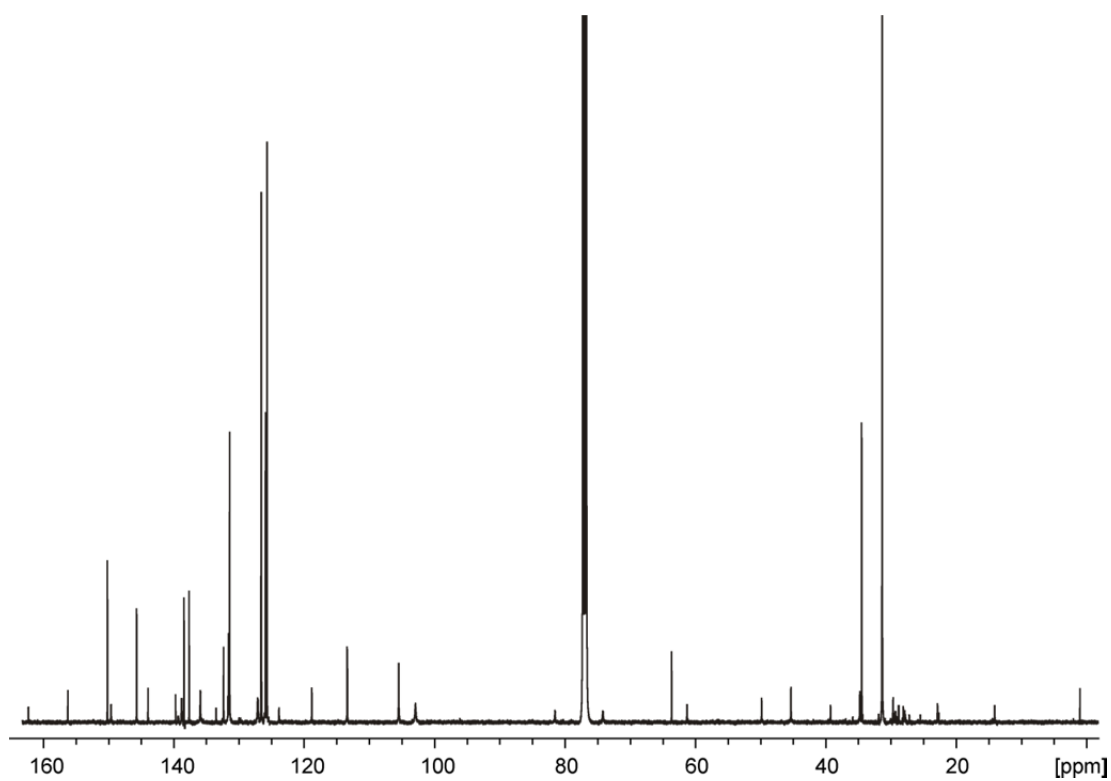


Figure S 10.  $^{13}\text{C}\{^1\text{H}\}$  NMR spectrum (125 MHz with cryoprobe,  $\text{CDCl}_3$ , 298 K) of a 1 mM solution of [2]rotaxane **5**.

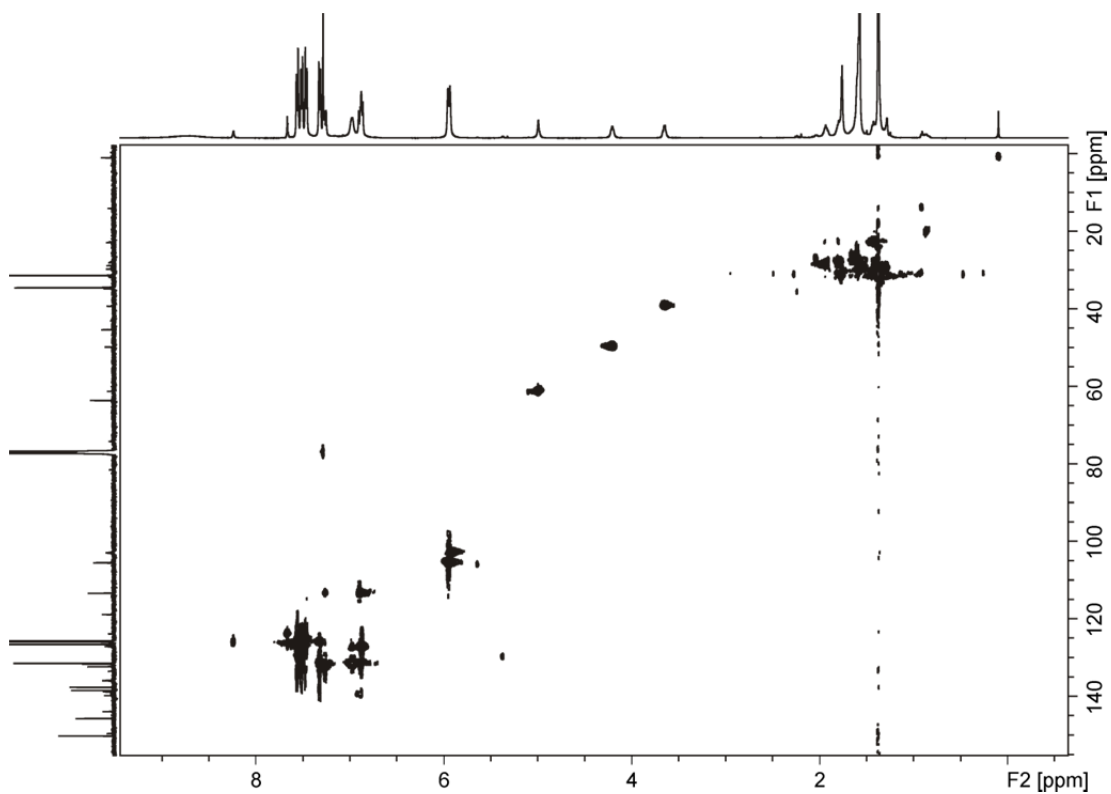


Figure S 11.  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum (125 MHz with cryoprobe,  $\text{CDCl}_3$ , 298 K) of a 1 mM solution of [2]rotaxane **5**.

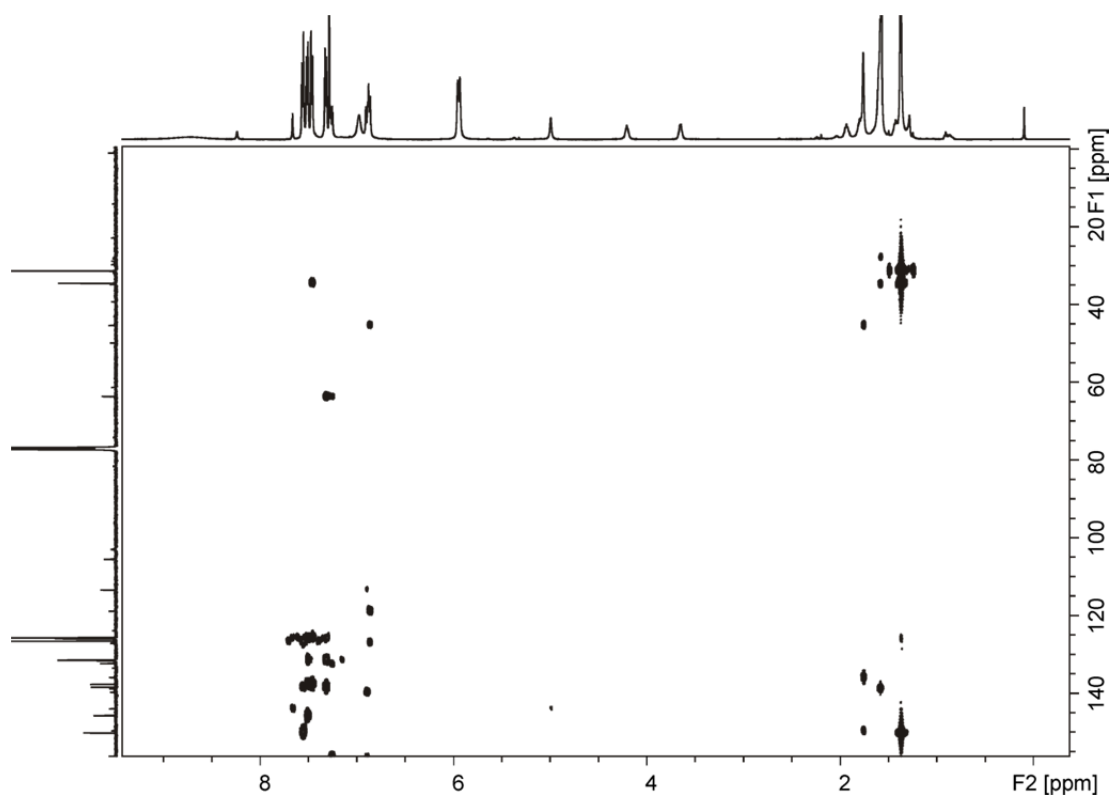


Figure S 12.  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum (125 MHz with cryoprobe,  $\text{CDCl}_3$ , 298 K) of a 1 mM solution of [2]rotaxane **5**.

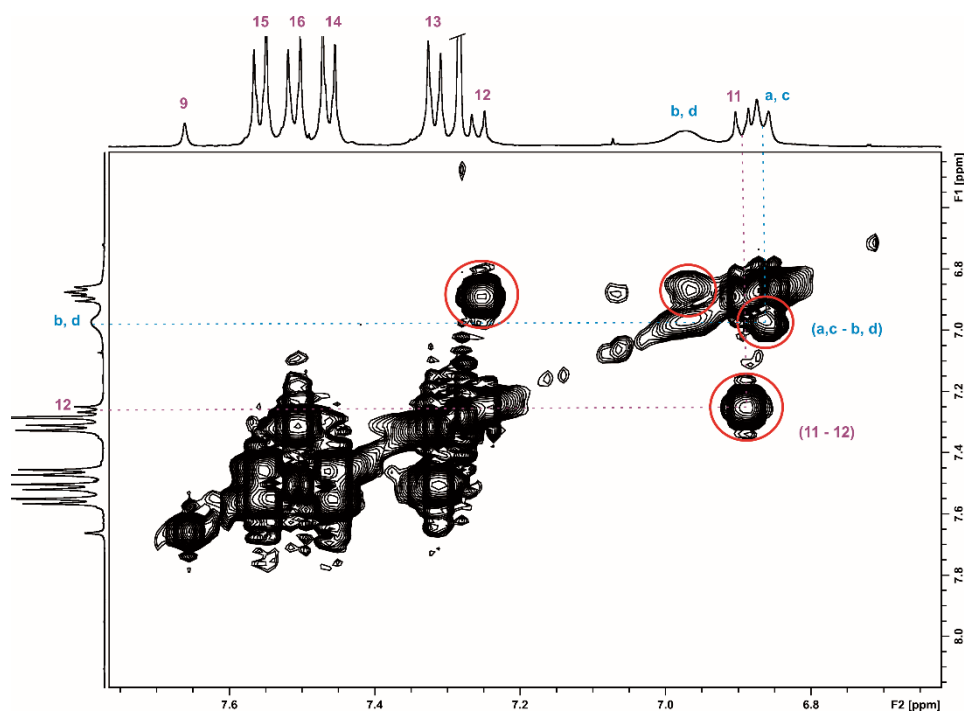


Figure S 13. Selected downfield region of the COSY experiment ( $\text{CDCl}_3$ , 500MHz, 298K) of a 1 mM solution of [2]rotaxane **5** showing the assignment of some relevant cross-peaks.

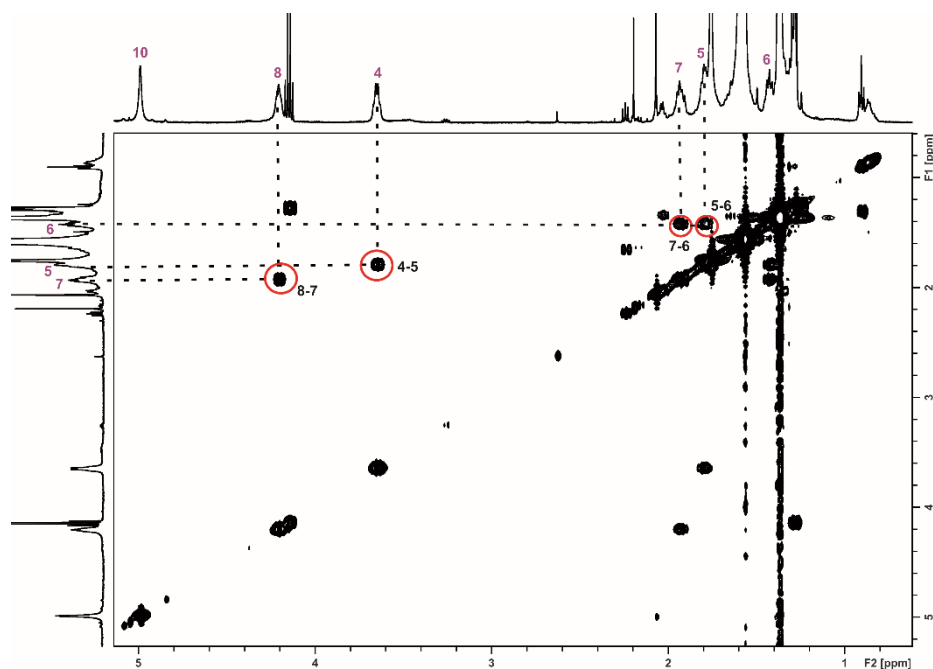


Figure S 14. Selected upfield region of the COSY experiment ( $\text{CDCl}_3$ , 500MHz, 298K) of a 1 mM solution of [2]rotaxane **5** showing the assignment of some relevant cross-peaks from the aliphatic chain of the linear component.

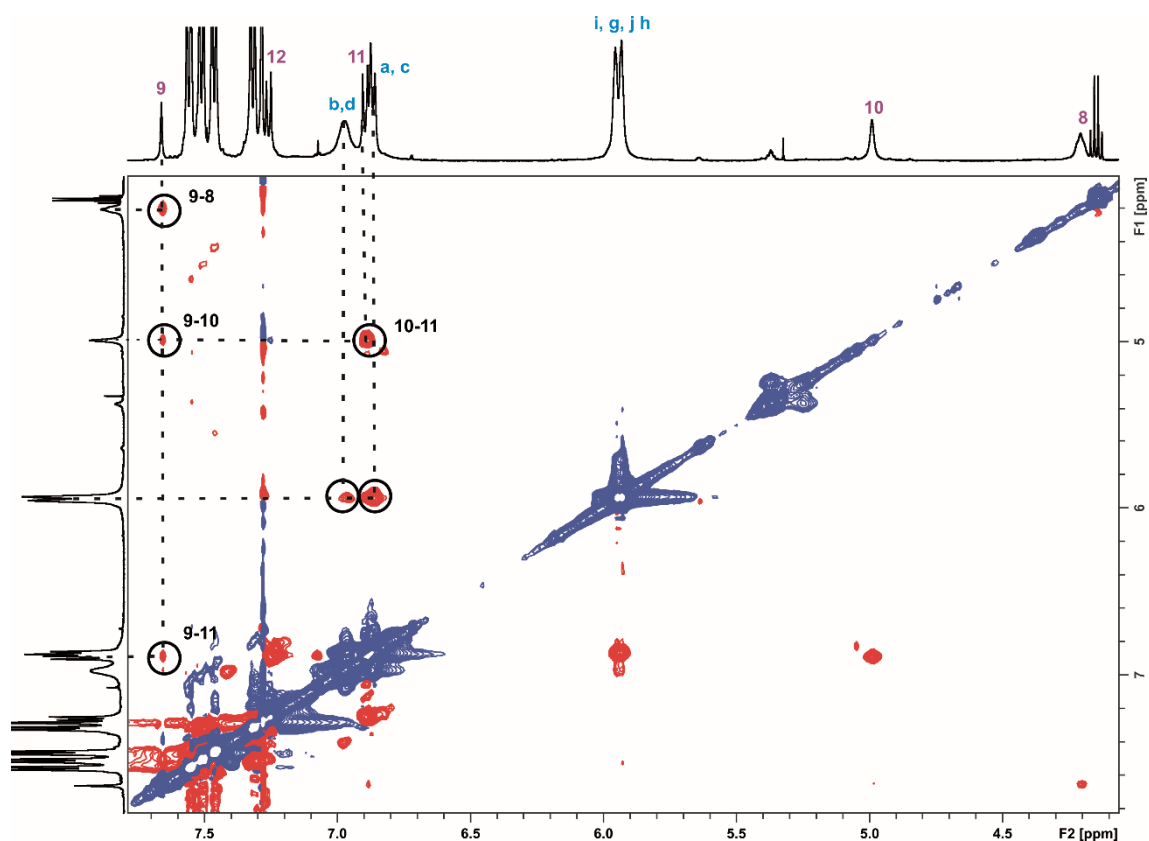


Figure S 15. Selected region of the ROESY experiment ( $\text{CDCl}_3$ , 500MHz, 298K) of a 1 mM solution of [2]rotaxane **5** showing the assignment of some relevant cross-peaks.

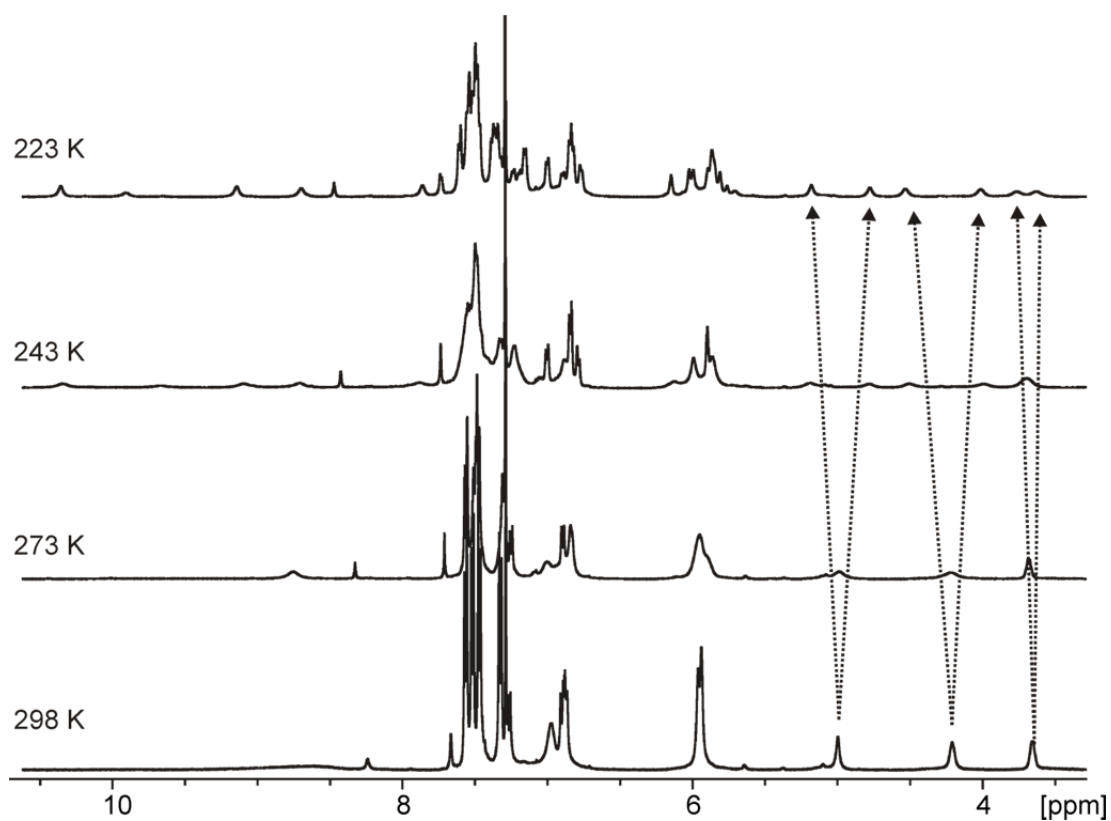


Figure S 16. Selected downfield region of the  $^1\text{H}$  NMR variable temperature (from 298 K to 223 K) experiment of a 1 mM solution of [2]rotaxane **5**.

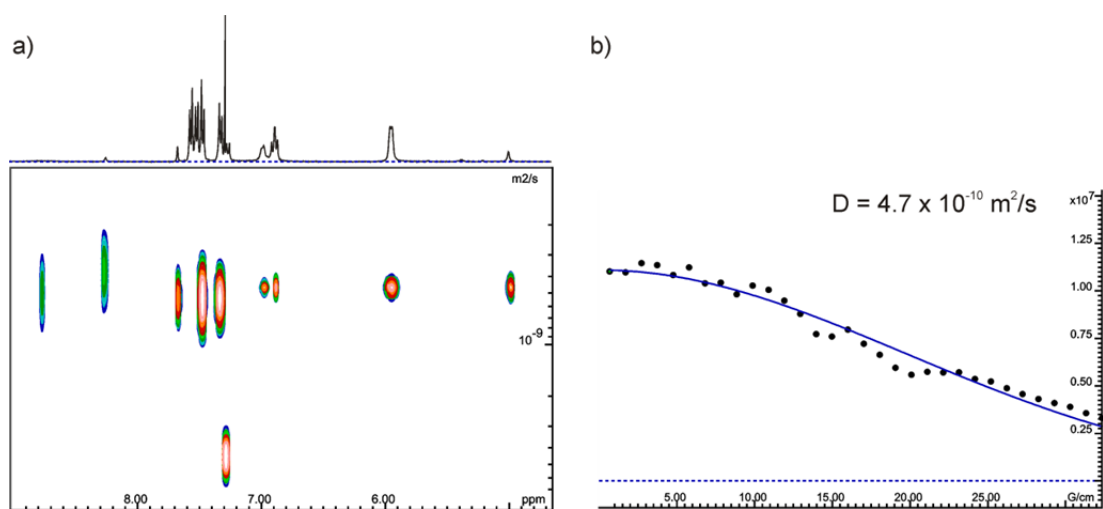


Figure S 17.  $^1\text{H}$  pseudo-2D DOSY ( $\text{CDCl}_3$ , 500MHz, 298 K) of a millimolar solution of [2]rotaxane **5** (a) and fit of the decay in the peak height at 4.99 ppm to a monoexponential function (b).

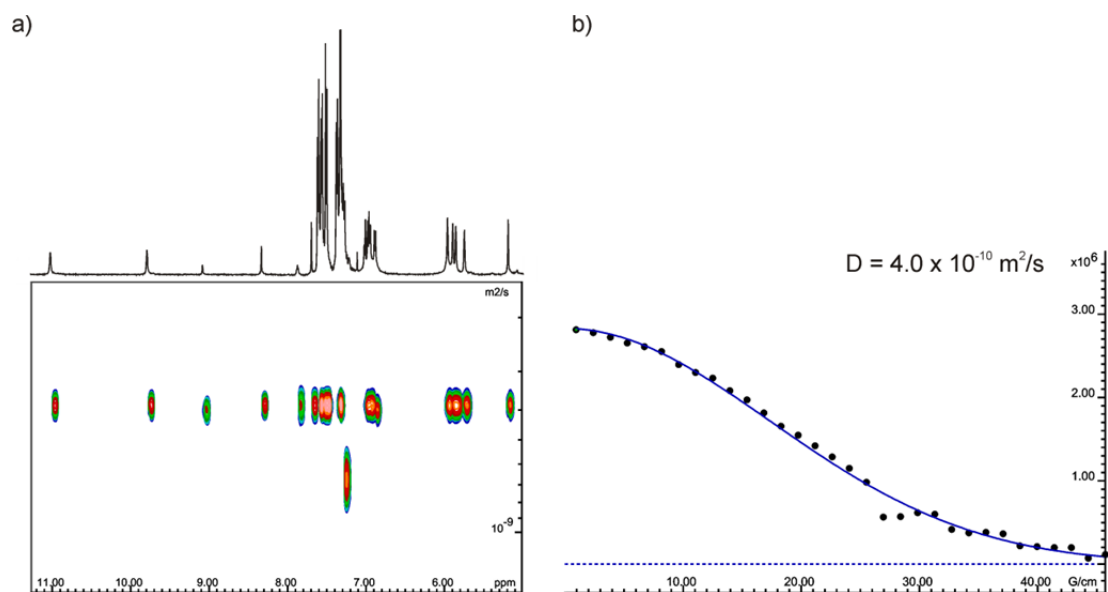


Figure S 18.  $^1\text{H}$  pseudo-2D DOSY ( $\text{CDCl}_3$ , 500MHz, 298 K) of a millimolar solution of an equimolar mixture of **3a** and [2]rotaxane **5** (a) and fit of the decay in the peak height at 5.17 ppm to a monoexponential function (b).

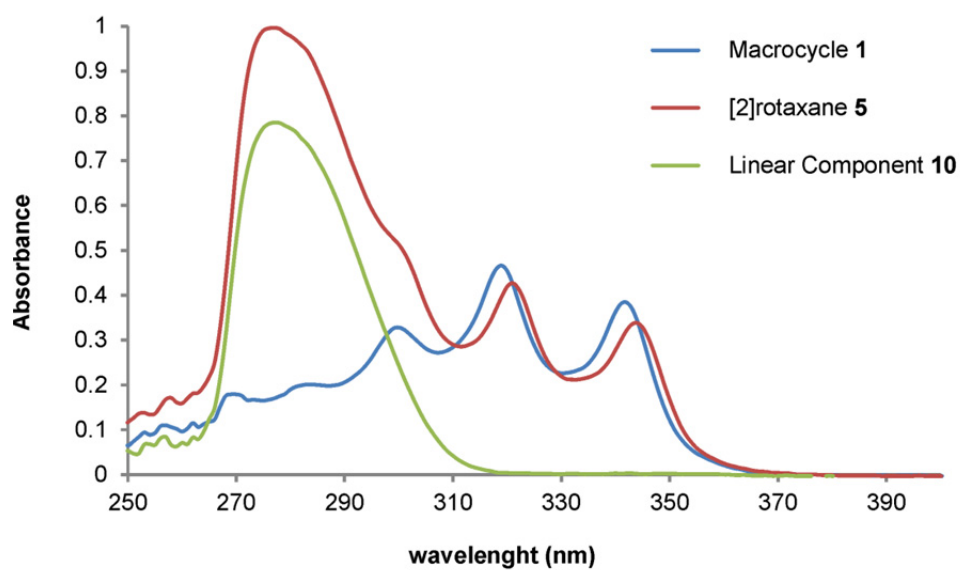


Figure S 19. UV-visible spectrum of a micromolar DCM solution of free macrocycle **1** (blue line), linear component **10** (green line) and [2]rotaxane **5** (red line).

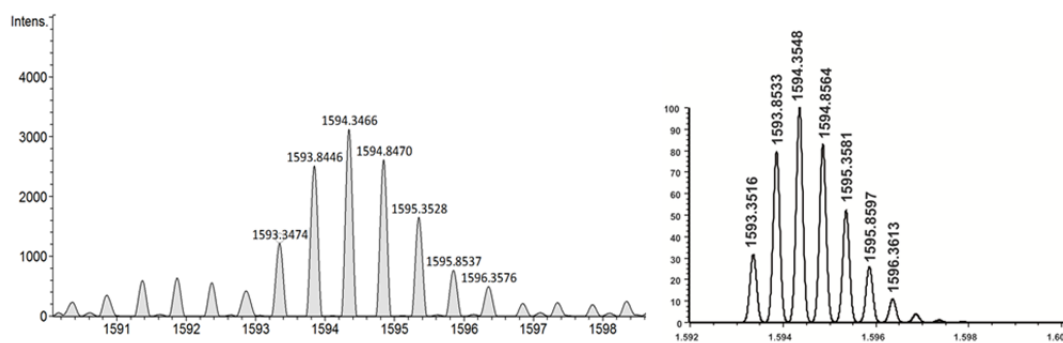


Figure S 20. Experimental (left) and theoretical (right) isotopic pattern of HR-MS spectrum of [2]rotaxane **5**.

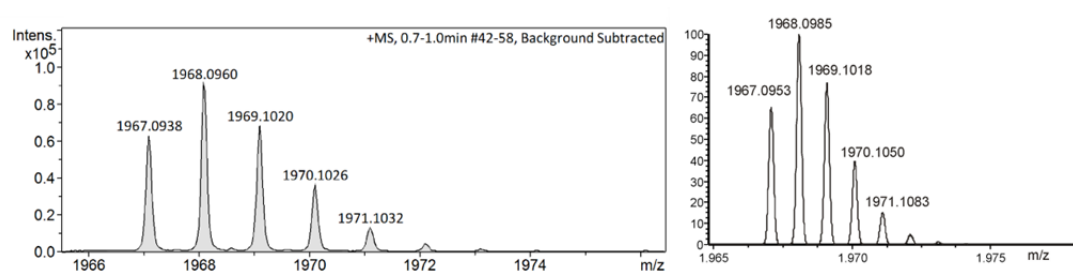


Figure S 21. Experimental (left) and theoretical (right) isotopic pattern of HR-MS spectrum of: linear component **10**.

### <sup>1</sup>H NMR titrations of [2]rotaxane in CDCl<sub>3</sub>

All titrations were carried out on a Bruker 400 MHz spectrometer using millimolar solutions of [2]rotaxane **5** in CDCl<sub>3</sub> at 298 K, and adding aliquots of a solution of the tetraalkylammonium salt (**3a**, **3b**, **3c** and **3d**), approximately 10 times more concentrated, in the same solvent. The rotaxane concentration was kept constant throughout the titration.

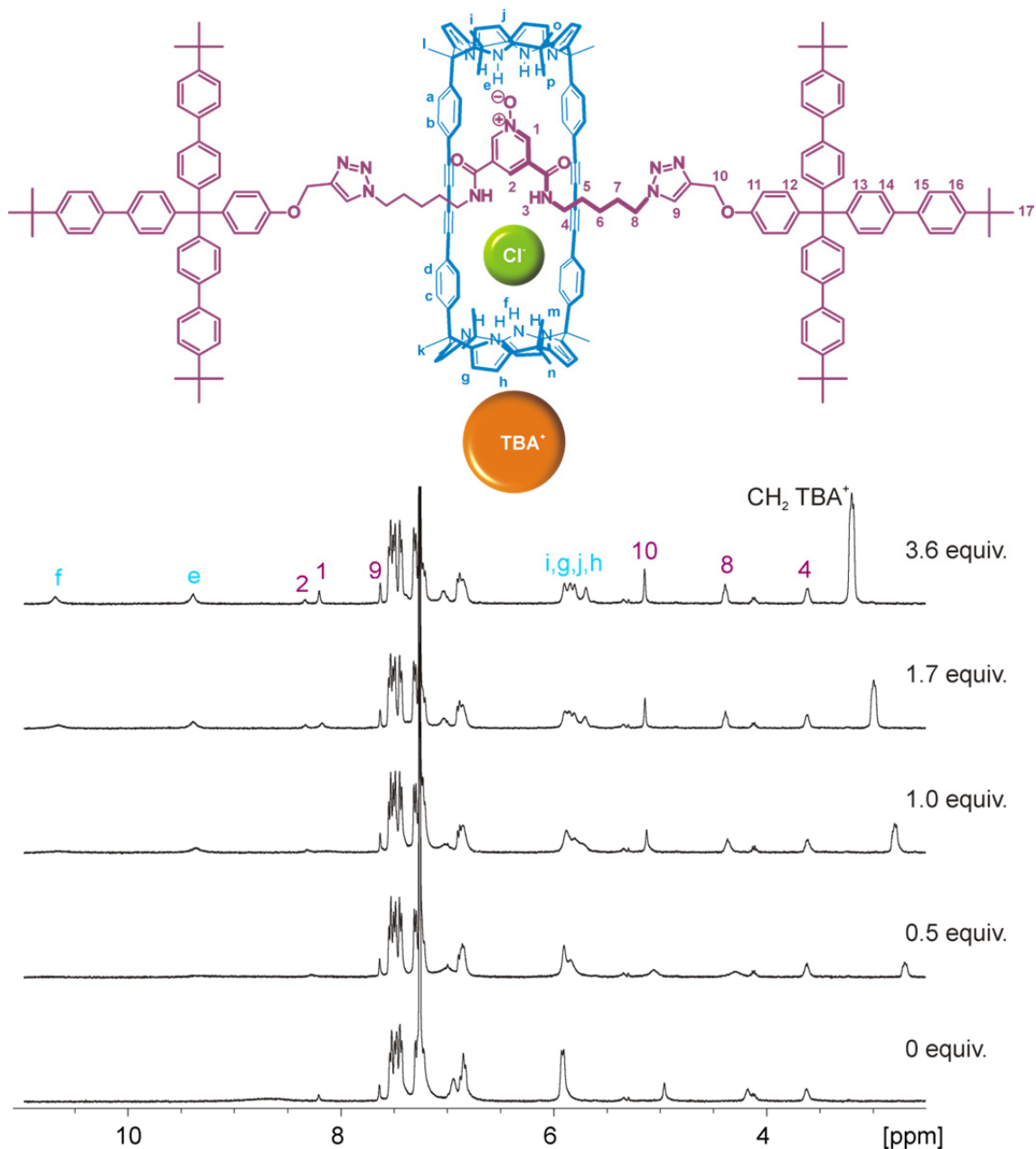


Figure S 22. Selected region of the  $^1\text{H}$  NMR titration ( $\text{CDCl}_3$ , 400MHz, 298 K) of a millimolar solution of [2]rotaxane **5** with successive additions of tetrabutylammonium chloride (**3c**) (from 0 to 3.6 equiv.).

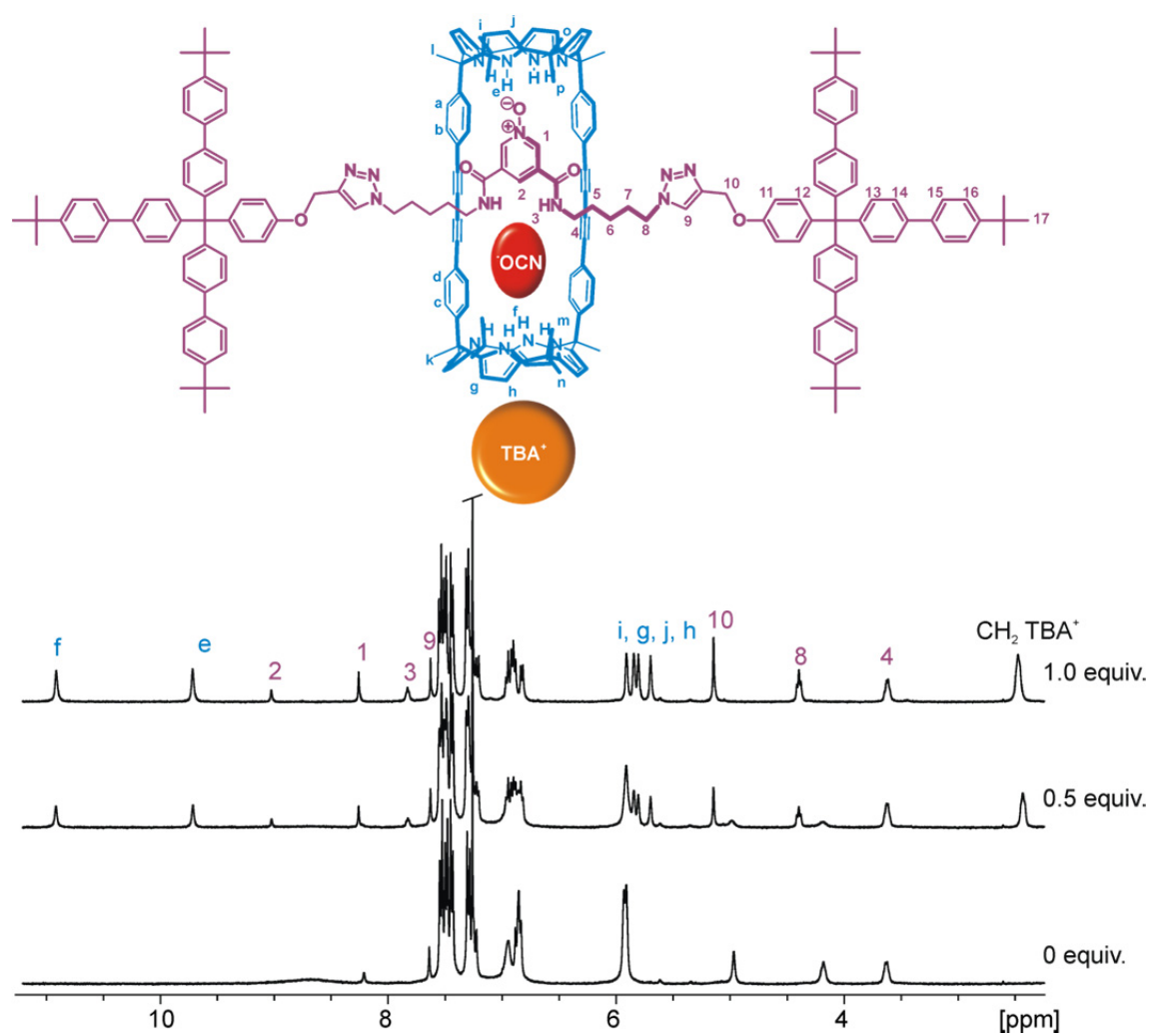


Figure S 23. Selected region of the  $^1\text{H}$  NMR titration ( $\text{CDCl}_3$ , 400MHz, 298 K) of a millimolar solution of [2]rotaxane **5** with successive additions of tetrabutylammonium cyanate (**3a**).

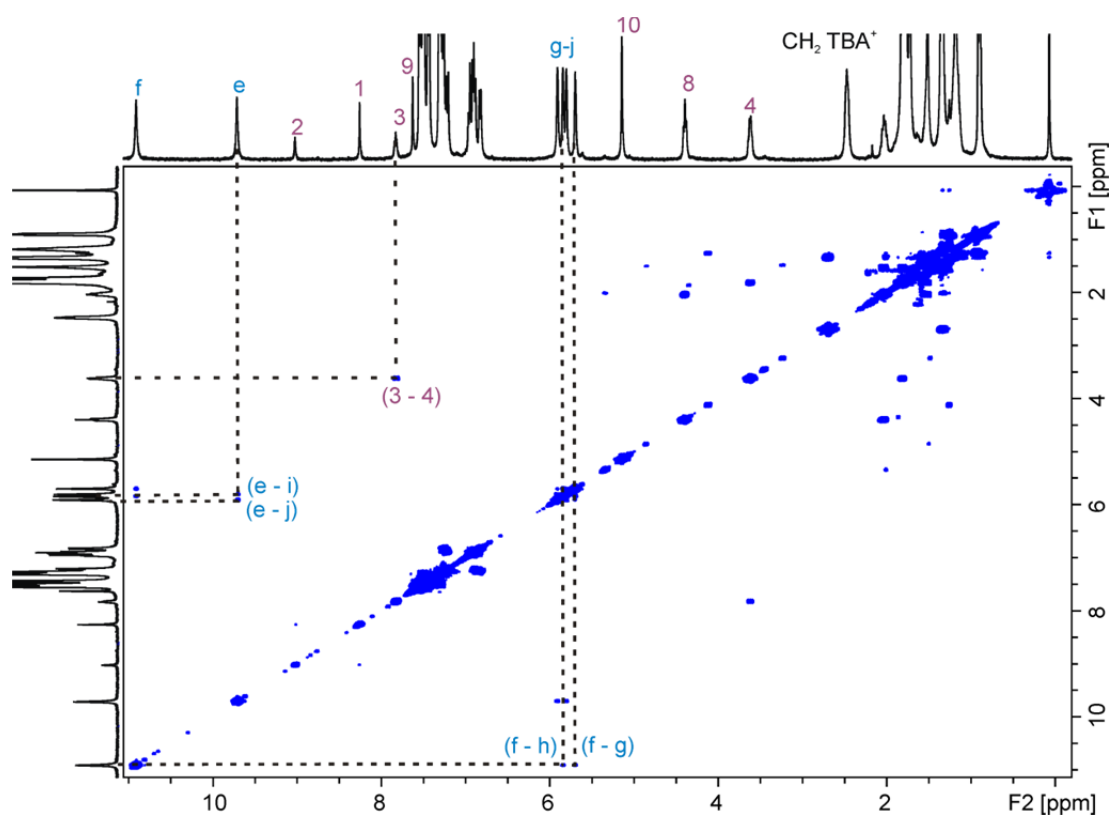


Figure S 24. COSY NMR experiment ( $\text{CDCl}_3$ , 500MHz, 298 K) of a millimolar solution of an equimolar mixture of [2]rotaxane **5** and tetrabutylammonium cyanate (**3a**).

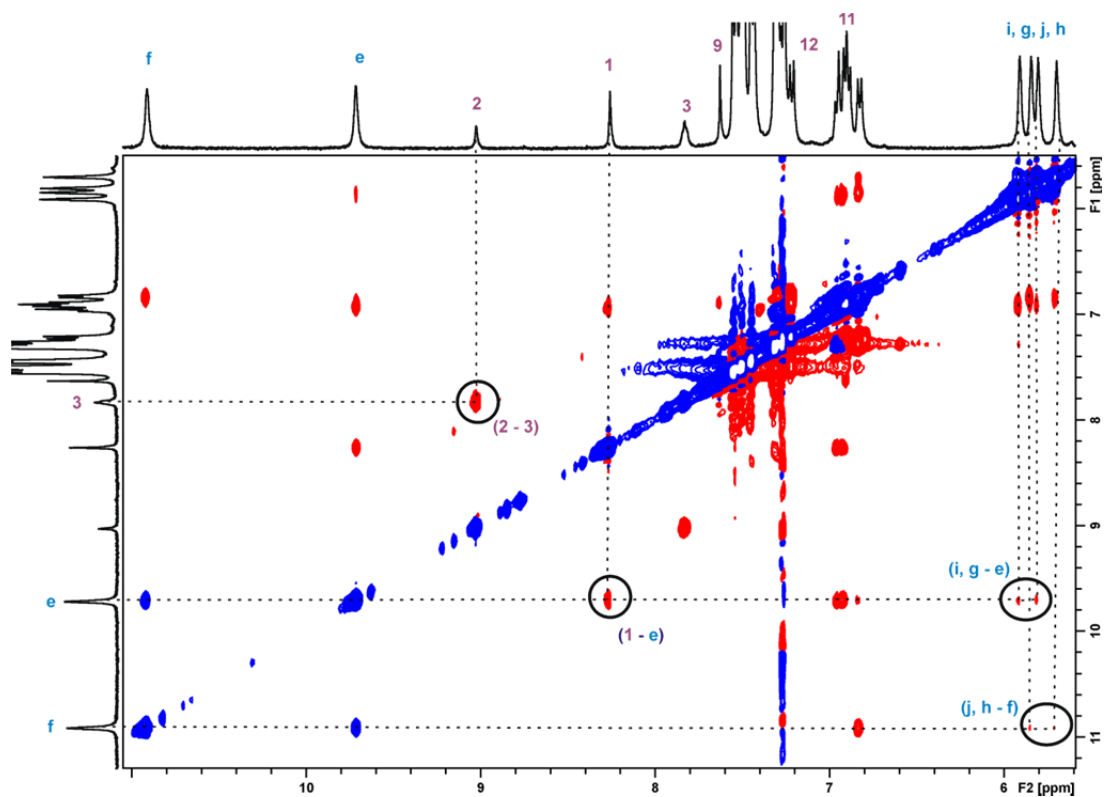


Figure S 25. Selected downfield region of the ROESY experiment ( $\text{CDCl}_3$ , 500MHz, 298 K) of a millimolar solution of an equimolar mixture of [2]rotaxane and tetrabutylammonium cyanate (**3a**). Some relevant cross-peaks have been highlighted.

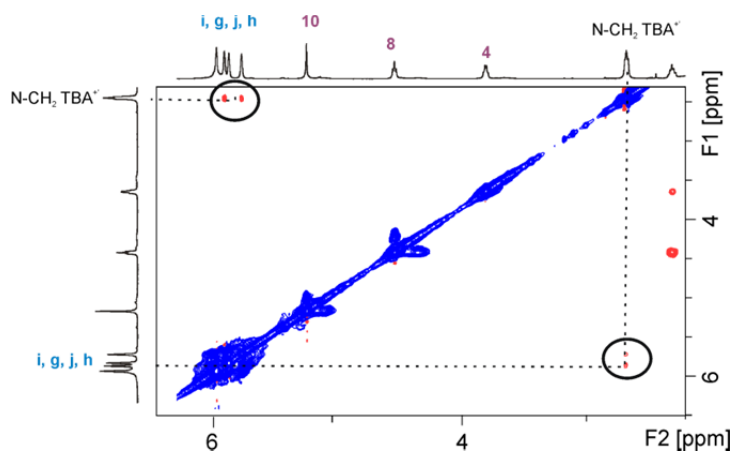


Figure S 26 Selected upfield region of the ROESY experiment ( $\text{CDCl}_3$ , 500MHz, 298 K) of a millimolar solution of an equimolar mixture of [2]rotaxane and tetrabutylammonium cyanate (**3a**). Some relevant cross-peaks have been highlighted.

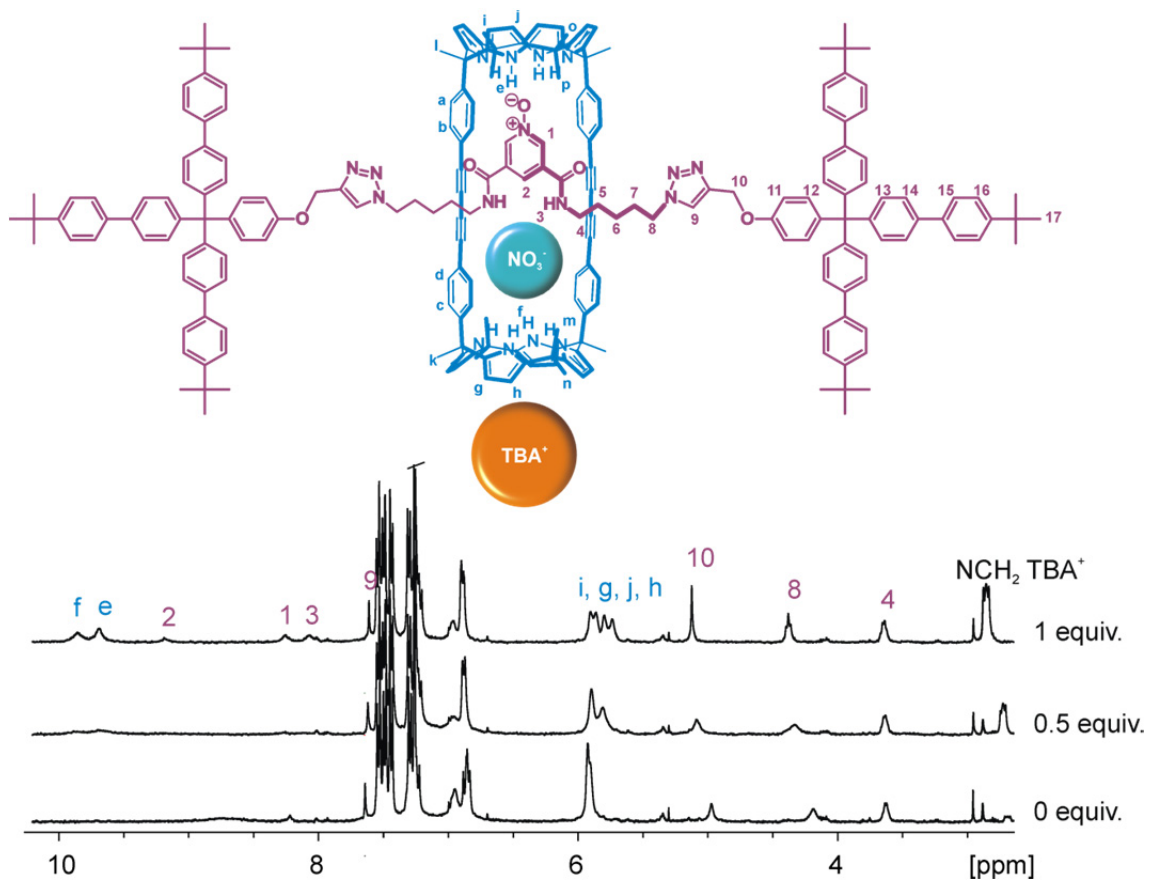


Figure S 27. Selected region of the  $^1\text{H}$  NMR titration ( $\text{CDCl}_3$ , 400MHz, 298 K) of a millimolar solution of [2]rotaxane **5** with successive additions of tetrabutylammonium nitrate (**3b**).

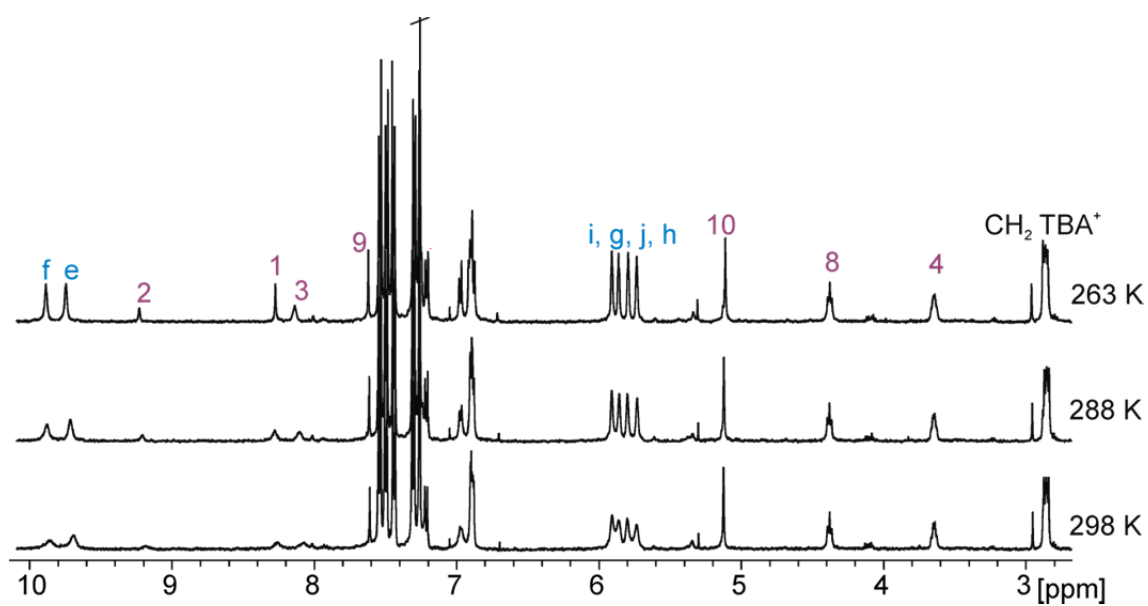


Figure S 28. Selected region of the variable temperature  $^1\text{H}$  NMR experiments ( $\text{CDCl}_3$ , 500MHz) performed on an equimolar mixture of [2]rotaxane and tetrabutylammonium nitrate (**3b**).

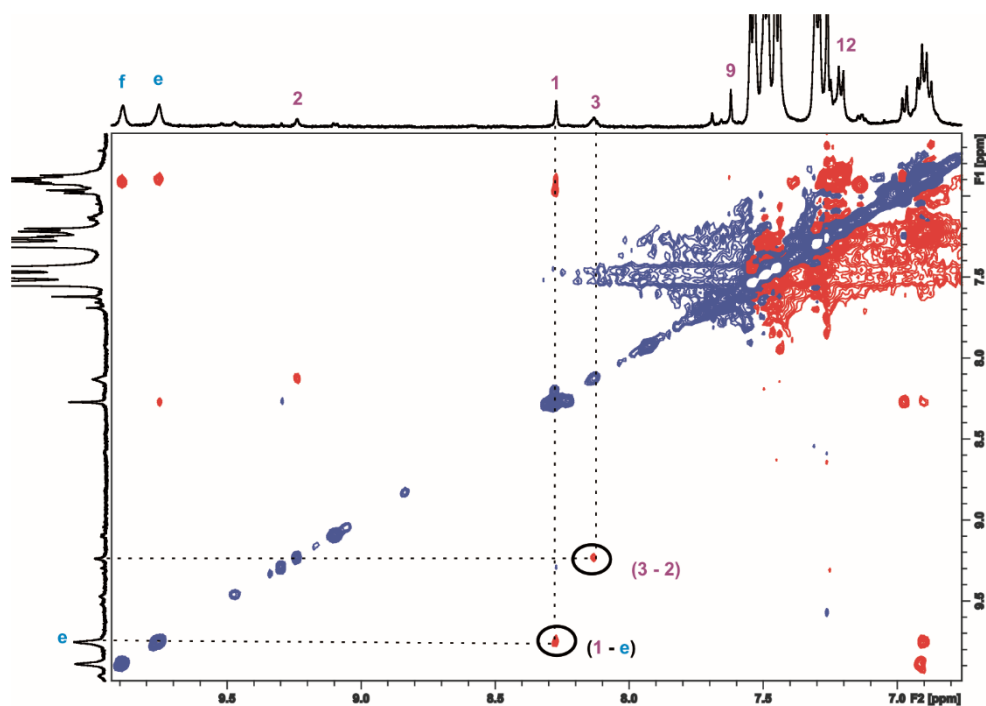


Figure S 29. Selected region of the ROESY experiment ( $\text{CDCl}_3$ , 500MHz) performed on an equimolar mixture of [2]rotaxane and tetrabutylammonium nitrate (**3b**) at 263 K showing the most relevant close contact peaks.

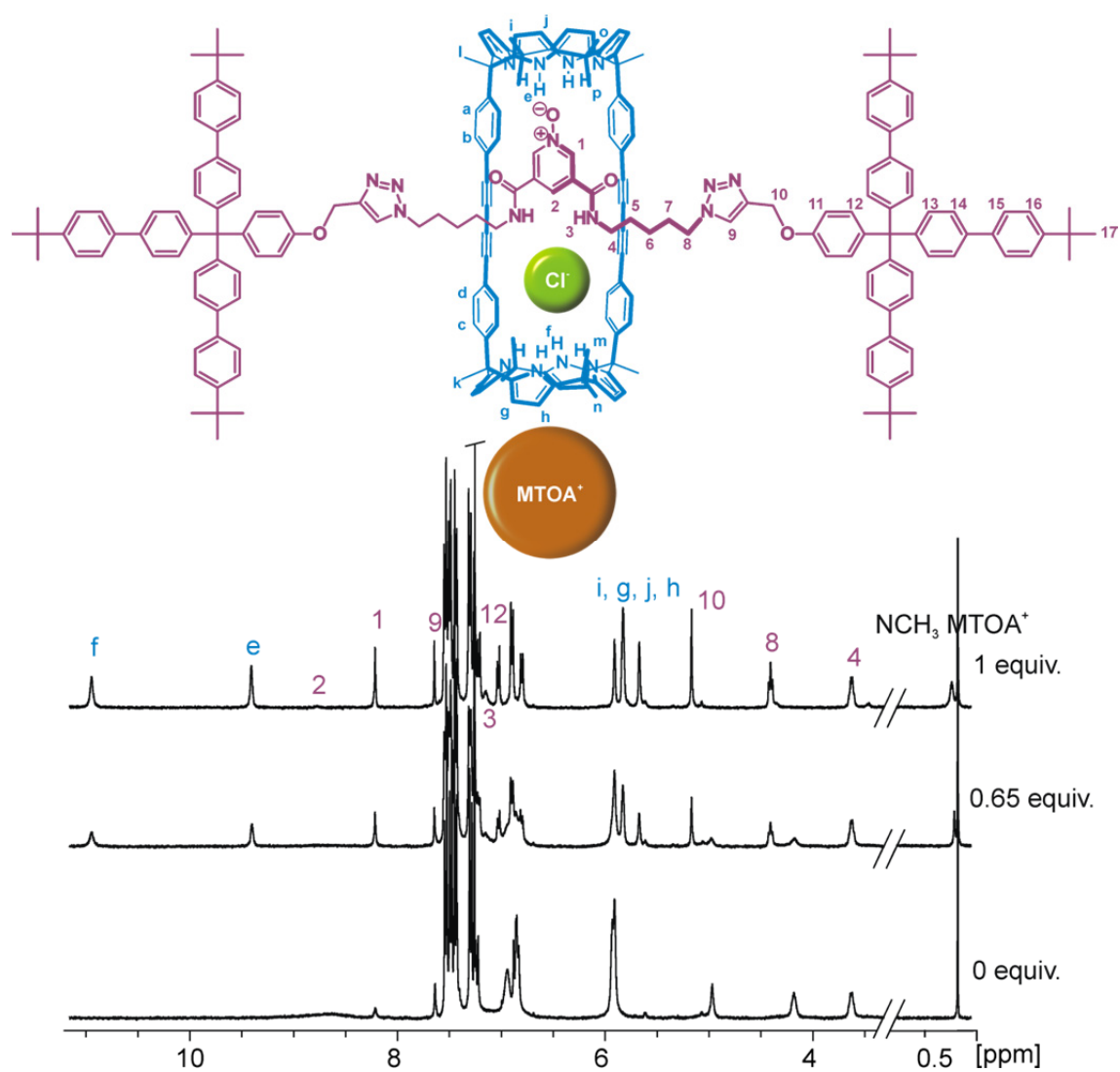


Figure S 30. Selected region of the  $^1\text{H}$  NMR titration ( $\text{CDCl}_3$ , 400MHz, 298 K) of a millimolar solution of [2]rotaxane **5** with successive additions of methyltriocetylammmonium chloride (**3d**).

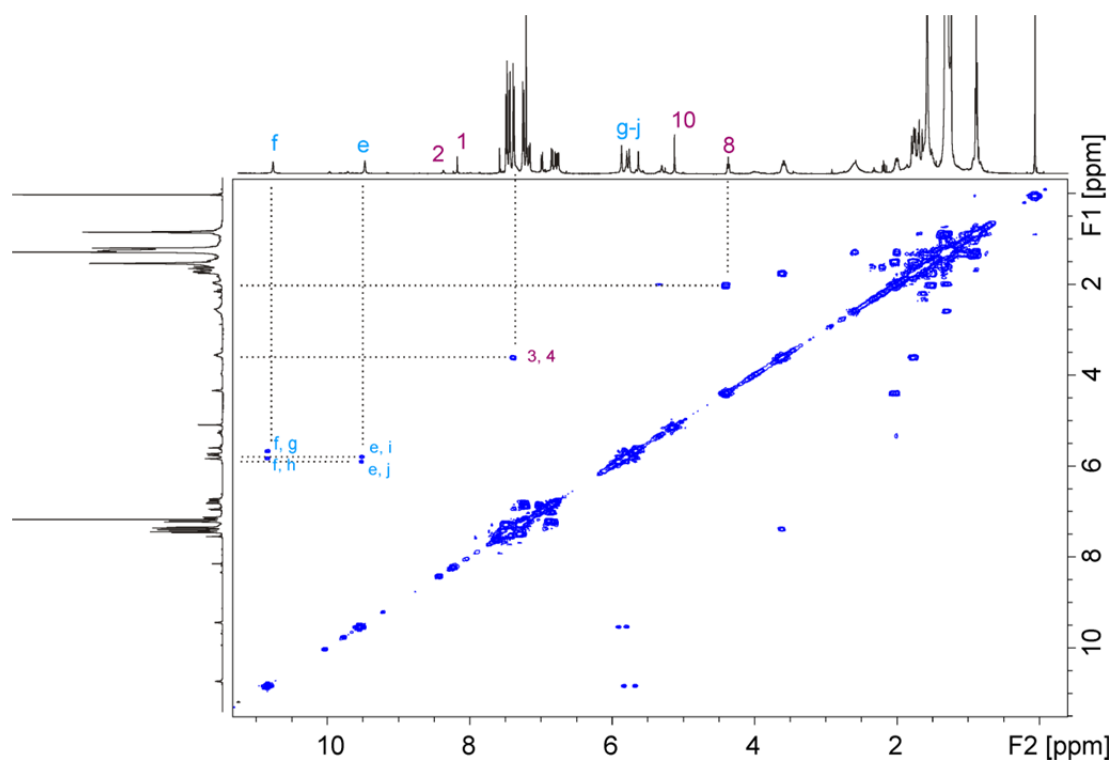


Figure S 31. COSY NMR experiment ( $\text{CDCl}_3$ , 500MHz, 298 K) of a millimolar solution of an equimolar mixture of [2]rotaxane **5** and methyltriocetylammmonium chloride (**3d**). Some relevant cross-peaks are highlighted.

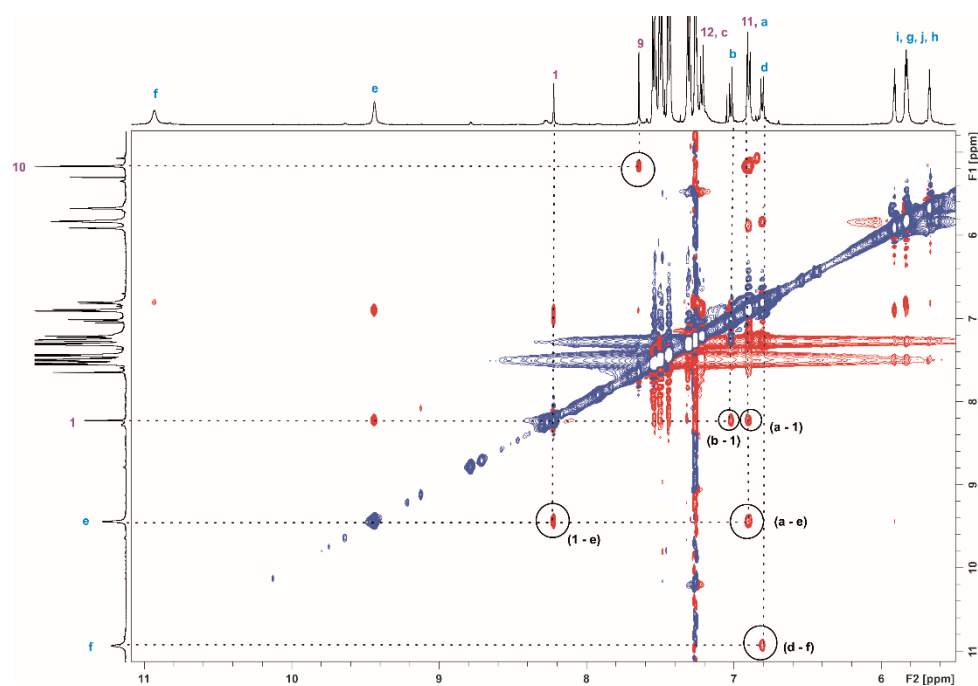


Figure S 32. Selected region of the T-ROESY experiment ( $\text{CDCl}_3$ , 500MHz, 263 K) of an equimolar mixture of [2]rotaxane **5** and methyltriocetylammmonium chloride (**3d**) highlighting some relevant close-contact cross-peaks. T-ROESY experiments were preferred to classical ROESY experiments as this sequence provides reliable dipolar cross-peaks with a minimal contribution of scalar transfer.

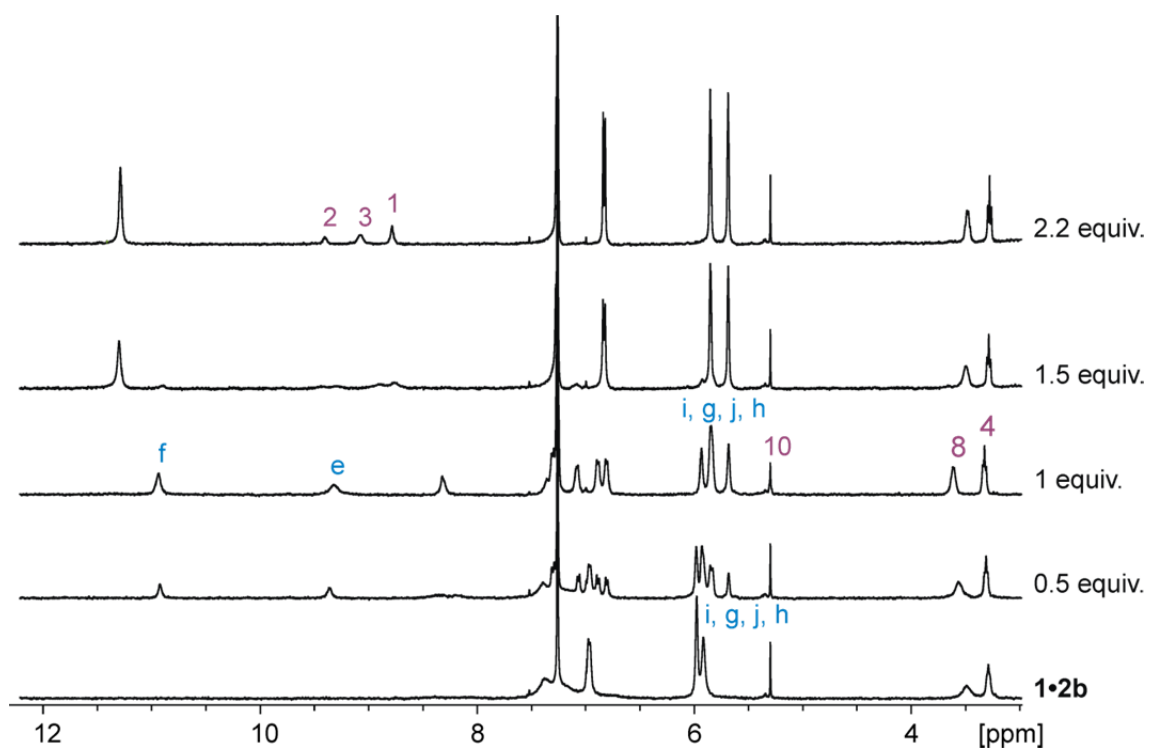


Figure S 33. Selected region of the  $^1\text{H}$  NMR titration ( $\text{CDCl}_3$ , 400 MHz, 298 K) of an equimolar solution of **1** and **2b** (1 mM) with successive additions of methyltrioctylammonium chloride **3d**.

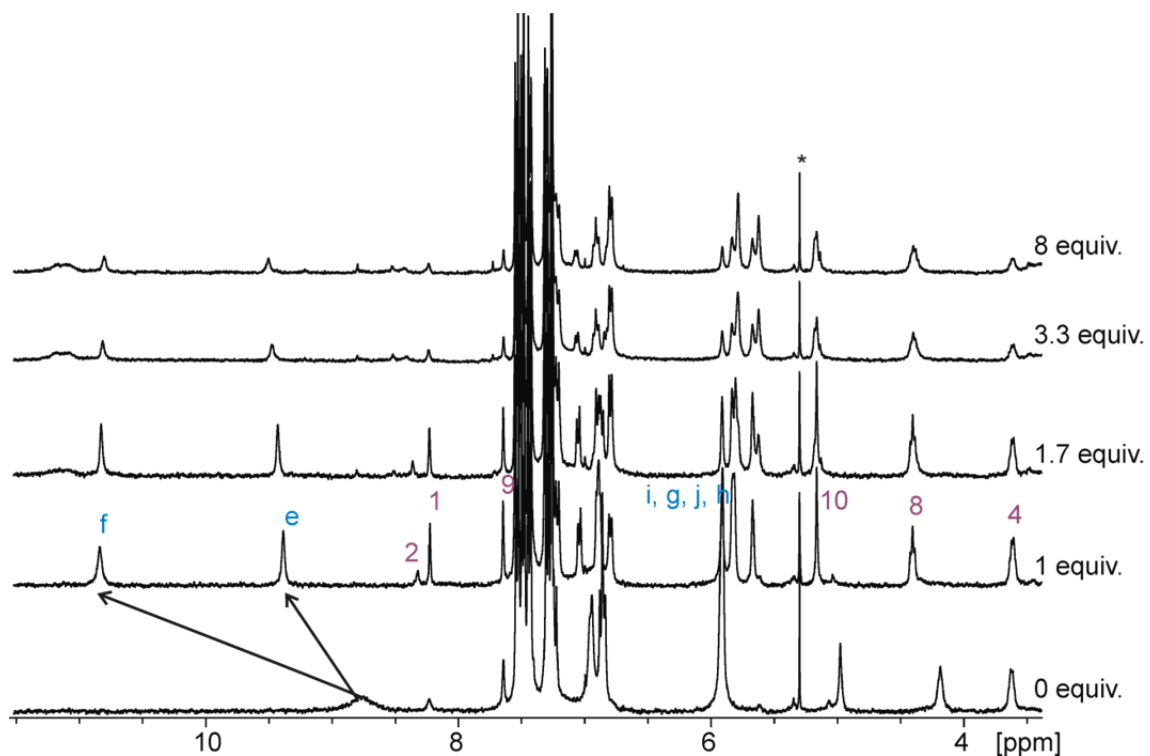


Figure S 34. Selected region of the  $^1\text{H}$  NMR titration ( $\text{CDCl}_3$ , 400 MHz, 298 K) of a solution of rotaxane **5** (1 mM) with successive additions of methyltrioctylammonium chloride **3d**.

## Isothermal Titration Calorimetry (ITC)

Isothermal titration calorimetry experiments were performed using a Microcal VP-ITC Microcalorimeter. All the titrations were carried out in chloroform solution at 298 K. Titrations of rotaxane **5** with tetraalkylammonium salts **3a-3d** were carried out by adding aliquots of a solution of the salt into a solution of rotaxane in the same solvent. The concentration of guests' solution was approximately 10 times more concentrated than the host one.

The association constants and the thermodynamic parameters were obtained from the fit of the titration data to a simple 1:1 binding model using the Microcal ITC Data Analysis module.

The low concentration used in the ITC experiments and the reduced excess of added tetraalkylammonium salts (up to 2 equiv.) made that the presence of species with stoichiometries higher than 1:1 was negligible.

The association constant ( $K_a$ ),  $T\Delta S$  and  $\Delta H$  values for the binding process were determined by averaging the values from two titrations.

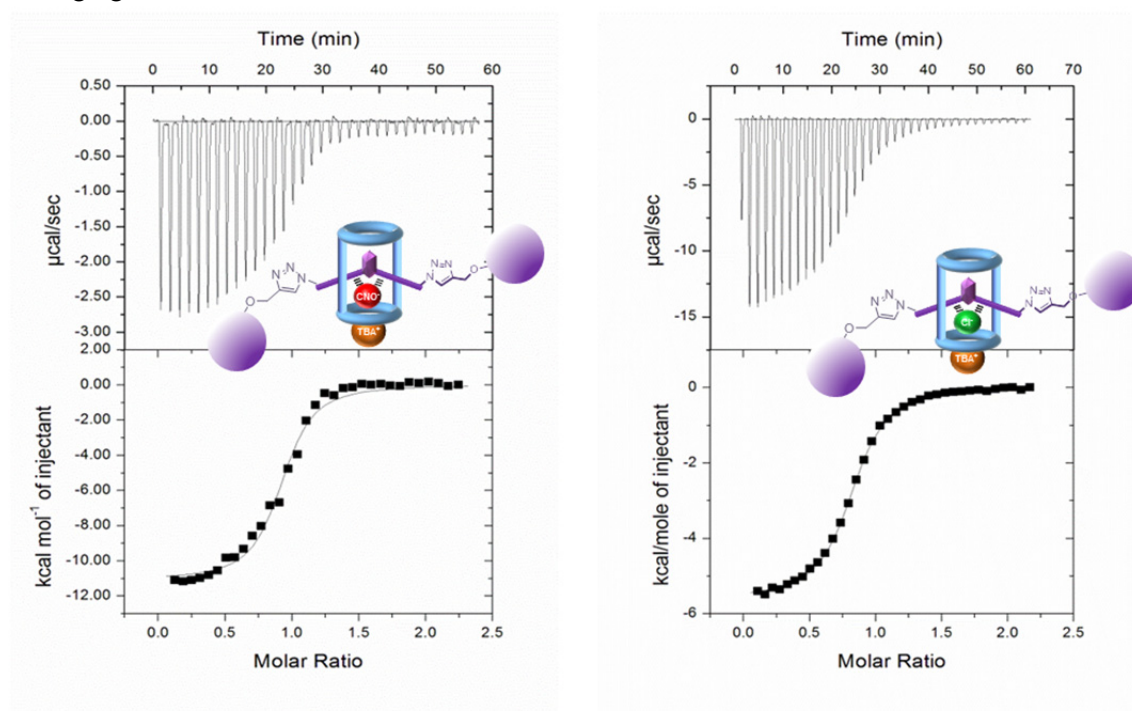


Figure S 35. Top – Trace of the raw data of the titration experiment of a  $5.8 \times 10^{-5}$  M and  $6.8 \times 10^{-4}$  M solution respectively, of rotaxane **5** with the tetrabutylammonium salts (TBACNO **3a** (left) and TBACl **3c** (right)) in  $\text{CHCl}_3$ . Bottom – Binding isotherm of the calorimetric titration shown on top. To determine the values of the thermodynamic variables the ITC data was fitted to a 1:1 binding model (black line).

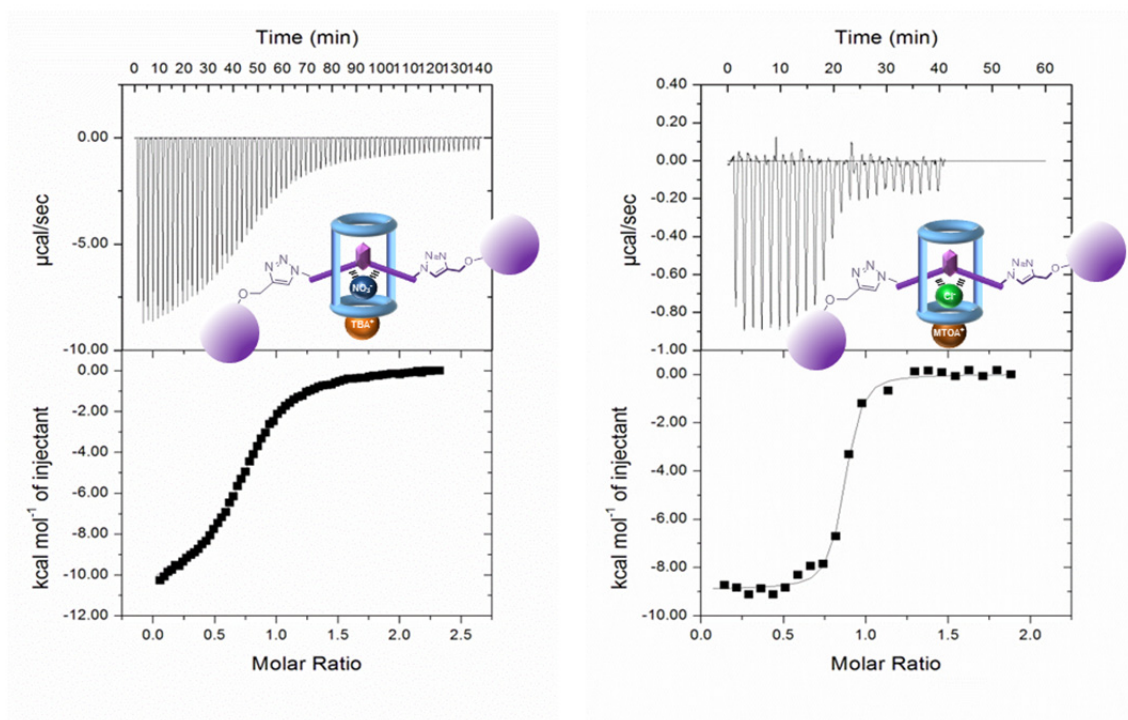


Figure S 36. Top – Trace of the raw data of the titration experiment of a  $5.2 \times 10^{-3}$  M and  $1.7 \times 10^{-4}$  M solution of rotaxane **5**, respectively with the tetraalkylammonium salts (TBANO<sub>3</sub> (left) and MTOACl (right)) in CHCl<sub>3</sub>. Bottom – Binding isotherm of the calorimetric titration shown on top. To determine the values of the thermodynamic variables the ITC data was fitted to a 1:1 binding model (black line).

## Simulated speciation profiles

Simulated speciation profiles of pseudorotaxane formation were obtained for different reaction conditions using the Hyss software. The stability constants used in the simulation were determined in previous works by  $^1\text{H}$  NMR and ITC experiments.

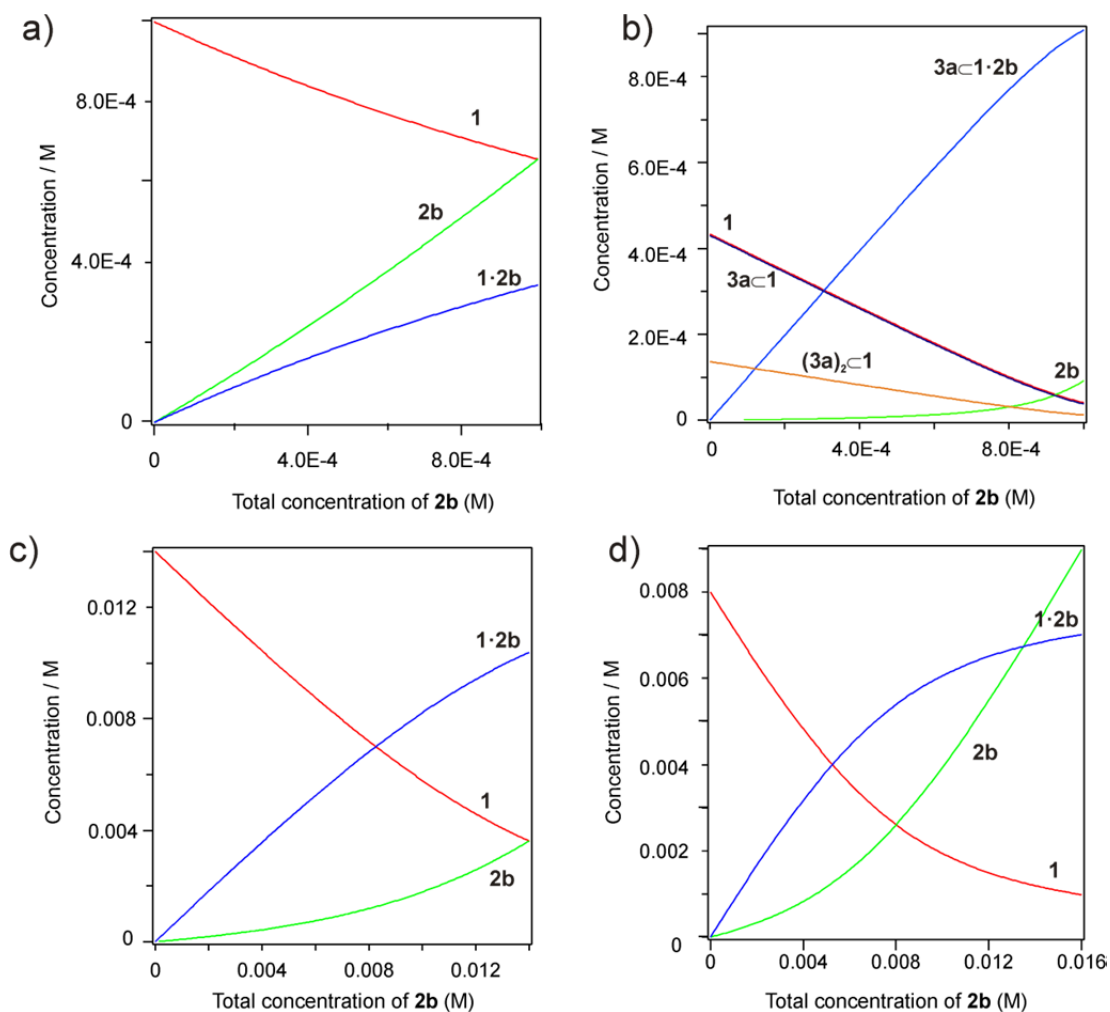


Figure S 37. Speciation profiles of the addition of increasing quantities of **2b** (up to 1 equiv.) over a 1 mM solution of macrocycle **1** (a), 1 mM equimolar mixture of **1** and **3a** (b), and 12 mM solution of macrocycle **1** (c). (d) Speciation profile of the addition of increasing quantities of **2b** (up to 2 equiv.) over a 8 mM solution of macrocycle **1**. We considered the association constants determined in previous works:  $K(\mathbf{1} \cdot \mathbf{2b}) = 800 \text{ M}^{-1}$ ,  $K(\mathbf{3a} \cdot \mathbf{1}) = 1 \times 10^5 \text{ M}^{-1}$ ,  $K(\mathbf{3a}_2 \cdot \mathbf{1}) = 1 \times 10^{11} \text{ M}^{-2}$ ,  $K(\mathbf{3a}_2 \cdot \mathbf{1} \cdot \mathbf{2b}) = 9.1 \times 10^{10} \text{ M}^{-2.5}$ .

## References

1. Thomas, Jason R.; Liu, Xianjun; Hergenrother, Paul J. *J. Am. Chem. Soc.* **2005**, *127*(36), 12434-12435
2. Wang, Hong; Zhu, Yuejun; Ren, Xiaona; Zhang, Hong; Tan, Yebang *Colloid and Polymer Science* **2012**, *290*(11), 1065-1075
3. Careful attention should be taken in the handling and storage of diazide **6** owing to its rather low molecular weight.
4. Clever, Guido H.; Shionoya, Mitsuhiro *Chem. Eur. J.* **2010**, *16*(39), 11792-11796
5. Valderrey, Virginia; Escudero-Adan, Eduardo C.; Ballester, Pablo *J. Am. Chem. Soc.* **2012**, *134*(26), 10733-10736